

Diversity of the genus *Tropodiaptomus* Kiefer, 1932 (Crustacea, Copepoda, Calanoida, Diaptomidae) in Thailand, with the description of two new species

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<https://zoobank.org/F8AA89A2-56BC-4A96-9835-C16AAE2744C1>

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Academic editor: Kay Van Damme ♦ Received 26 April 2023 ♦ Accepted 16 June 2023 ♦ Published 5 July 2023

Abstract

Tropodiaptomus is a genus of diaptomid copepods with 10 species currently recorded in Thailand. A recent study on DNA taxonomy revealed putative new species among specimens collected from freshwater habitats throughout Thailand. This study examined the morphological characteristics and confirmed the taxonomic status of the two putative new species of *Tropodiaptomus*. Results showed that the two new taxa were different from other species in the genus *Tropodiaptomus*. These two new species, *T. pedecrassum* sp. nov. and *T. longiprocessus* sp. nov., were described and illustrated based on material collected from a swamp in northern Thailand and a pond in western Thailand, respectively. They were distinguished from their congeners by the length of the spinous process on the antepenultimate segment of the adult male right antennule, the number of lobes and serration pattern on the inner margin of the adult male left P5, and the shape and supplementary process on the surface structures of basis and distal exopod segments of the adult male right P5. These discoveries increased the number of records of this genus in Thailand to 12 species. A pictorial key to all species is provided, and their ecological and biogeographical distributions are updated and discussed.

Key Words

habitat preference, new species, Thailand, *Tropodiaptomus longiprocessus*, *Tropodiaptomus pedecrassum*

Introduction

To date, 170 copepod species have been recorded from freshwater habitats in Thailand (Saetang et al. 2020; Sanoamuang and Dabseepai 2021). The diaptomid family in the calanoid group is highly diverse, comprising 42 species in Thailand, with 14 new species added during the last 20 years. *Mongolodiaptomus* and *Tropodiaptomus* are the two most diverse genera, each containing 10 species (Saetang et al. 2020; Sanoamuang and Dabseepai 2021). Interestingly, *Tropodiaptomus* has the highest number of 63 species in the family Diaptomidae worldwide (Walter and Boxshall 2023). Most species in this genus are restricted to one or a few biogeographic regions, with 21 species distributed in Asia

(Saetang et al. 2020; Walter and Boxshall 2023). In Thailand, undiscovered species are postulated to exist within specific habitats such as temporary ponds and this genus remains underexplored. Saetang et al. (2020) investigated 468 samples collected from 190 freshwater habitats throughout Thailand. Eight species were reported with one new to science. Later, Saetang (2021) and Saetang et al. (2022) conducted extensive morphological and DNA taxonomy studies on *Tropodiaptomus*, with results determining at least 12 species recorded in Thailand. These integrative methods indicated a high level of genetic diversity in some *Tropodiaptomus* species, with the possibility of cryptic species. Based on genetic data, three more putative new species await morphological study and description (Saetang et al. 2022).

Our study proposes *Tropodiaptomus* sp. 1 and *Tropodiaptomus* sp. 2 mentioned in Saetang et al. (2022) as new species with detailed descriptions and illustrations of both males and females. A pictorial key for practical use to identify members of this genus was presented and the present diversity and species distribution were updated.

Materials and methods

Samples were qualitatively collected from swamp in Dok Kham Tai District, Phayao Province, northern Thailand ($19^{\circ}13'57.6''N$, $100^{\circ}02'56.5''E$) in January 2018 and Thong Phaphum District, Kanchanaburi Province ($14^{\circ}39'09.1''N$, $98^{\circ}33'27.5''E$) in June 2019 using a plankton net of 60 μm mesh and immediately preserved in 70% ethanol. All adult males and females were sorted using an Olympus SZ40 stereo microscope, and each specimen was dissected and mounted on a slide in glycerine and then sealed using nail varnish.

The morphological characteristics were examined and identified using an Olympus CH2 compound microscope, and drawings were made from both complete and dissected specimens using a camera lucida connected to the microscope. The final versions of the drawings were made using Adobe Illustrator CS5 program (version 15.0). The specimens were identified to species level according to Lai et al. (1979), Lai and Fernando (1981), Kiefer (1982), and Sanoamuang (2002). The descriptive terminology proposed by Huys and Boxshall (1991) was adopted. Abbreviations used in the text and figures are: **A1** = antennule, **ae** = aesthetasc, **s** = spine, **sp** = spinous process, **A2** = antenna, **P1–P5** = swimming legs 1–5, **exp-1 (2, 3)** = first (second and third) segment of exopod, **enp-1 (2, 3)** = first (second and third) segment of endopod.

All type specimens were deposited in the reference collection of the Princess Maha Chakri Sirindhorn National History Museum, Prince of Songkla University, Songkhla, Thailand (PSUNHM).

Results

Systematics

Order CALANOIDA Sars, 1903

Family DIAPTOMIDAE Baird, 1850

Genus *Tropodiaptomus* Kiefer, 1932

Type species. *Tropodiaptomus orientalis* (Brady, 1886).

Tropodiaptomus pedecrassum sp. nov.

<https://zoobank.org/D922BBEE-6472-4B19-AF44-540A34C93DB4>

Type locality. Swamp near rice field, Dok Kham Tai District, Phayao Province, northern Thailand

($19^{\circ}13'57.6''N$, $100^{\circ}02'56.5''E$). Temporary habitat without macrophytes.

Material examined. **Holotype.** One adult male, dissected and mounted onto five slides, Dok Kham Tai, Phayao province, northern Thailand ($19^{\circ}13'57.6''N$, $100^{\circ}02'56.5''E$), 31 January 2018, Thanida Saetang and Supiyanit Maiphae; PSUZC-PK2008-01–PSUZC-PK2008-05.

Allotype. One adult female, collected with holotype; PSUZC-PK2008-06–PSUZC-PK2008-08.

Paratype. One adult male, collected with holotype; PSUZC-PK2008-09–PSUZC-PK2008-11.

Description of the adult male. **Body** (Figs 1A, 2A). Total body length about 1,145 μm (1.1 mm) (measured from anterior margin of rostrum to posterior margin of caudal rami). Prosome length about 2 times as long as urosome (including caudal rami). Fourth and fifth pedigers separated by distinct septum. Fifth pediger produced into small asymmetrical posterolateral wings (left wing shorter than right wing), each distal end with spine. Urosome 5-segmented. Genital somite with dorsolateral sensilla on right and left side, fourth somite with expanded right corner. Anal somite with deep cleft, length about 0.8 times as long as wide. Caudal rami parallel, symmetrical, length about 1.8 times as long as wide, with setules on inner margin. Each ramus with six setae.

Rostrum (Fig. 3A). Two rostral elements on anterior margin with suture in the middle.

A1 (Figs 1B, C, 3B–D). Asymmetrical. **Left A1** non-geniculate, 25-segmented, reaching beyond the end of caudal rami. Armature formula of each segment as follows: 1+ae, 3+ae, 1+ae, 1, 1+ae, 1, 1+ae, 1+s, 2+ae, 1, 1, 1+ae+s, 1, 1+ae, 1, 1+ae, 1, 1, 1+ae, 1, 1, 2, 2, 2, 5+ae. **Right A1** transformed and geniculate, 22-segmented. Strongly dilated between segment 13 and segment 18. Spinous process on segment 20 (antepenultimate) straight and bent at distal end, reaching 3/4 next segment, and with longitudinal hyaline membrane on outer margin (Fig. 7A–G, arrowhead). Armature formula of each segment as follows: 1+ae, 3+ae, 1+ae, 1, 1+ae, 1, 1+ae, 1+s, 2+ae, 1+sp, 1+sp, 1+ae+s, 1+ae+sp, 2+ae, 2+ae+sp, 2+ae+sp, 1+s, s, 1+3s, 4+sp, 2, 5+ae.

A2 (Fig. 4A). Coxa with one inner seta on distal corner. Basis with two inner setae on distal corner. Exopod 7-segmented, exp-1–6 with 1, 3, 1, 1, 1, and 1 inner setae, respectively, and exp-7 with one inner and three apical setae. Endopod 2-segmented, enp-1 with two inner setae and one longitudinal row of outer spinules, enp-2 with nine inner and seven apical setae; and one group of outer spinules.

Mandible (Fig. 4B). Coxa with eight strongly chitinised teeth and one seta on gnathobase. Basis with four inner setae. Exopod 4-segmented with 1, 1, 1, and 3 setae, respectively. Endopod 2-segmented, enp-1 with four inner setae, enp-2 with nine apical setae and one longitudinal row of outer spinules.

Maxillule (Fig. 4C). Precoxal arthrite with four plumose setae and eleven bipinnate spines.

Coxal endite with three plumose setae, and coxal epipodite with seven plumose setae and two bipinnate spines. Basis with two endites; the proximal with four

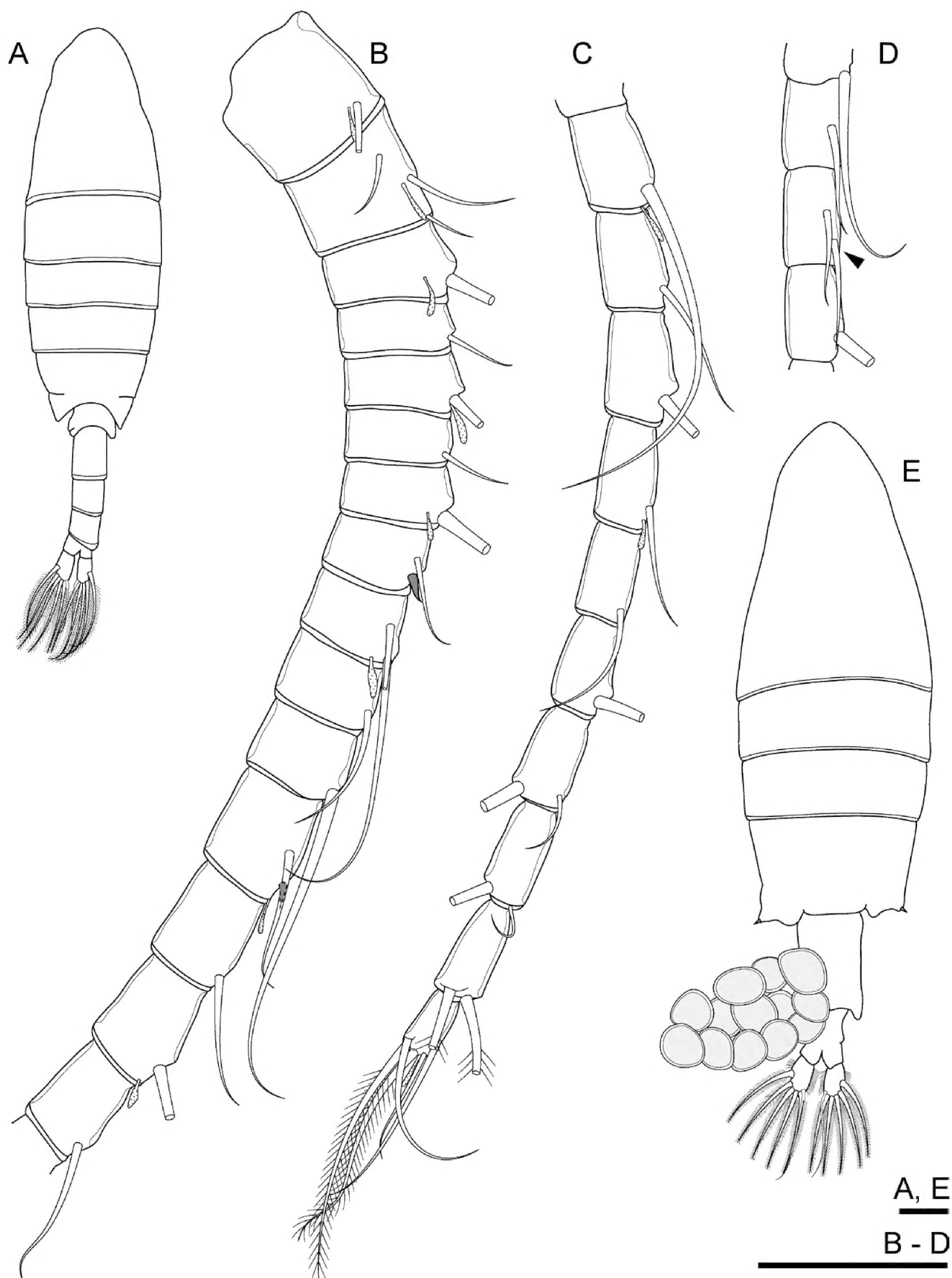


Figure 1. *Tropodiaptomus pedecrassum* sp. nov., male holotype. **A.** Habitus, dorsal view; **B.** Segment 1–15 of left antennule (gray color indicates spine); **C.** Segment 16–25 of left antennule; **D.** Segment 12–14 of left antennule of the adult male specimen from the same population of holotype, (arrowhead indicates variation of setae on segment 13); female allotype; **E.** Habitus, dorsal view. Scale bars: 100 μ m.

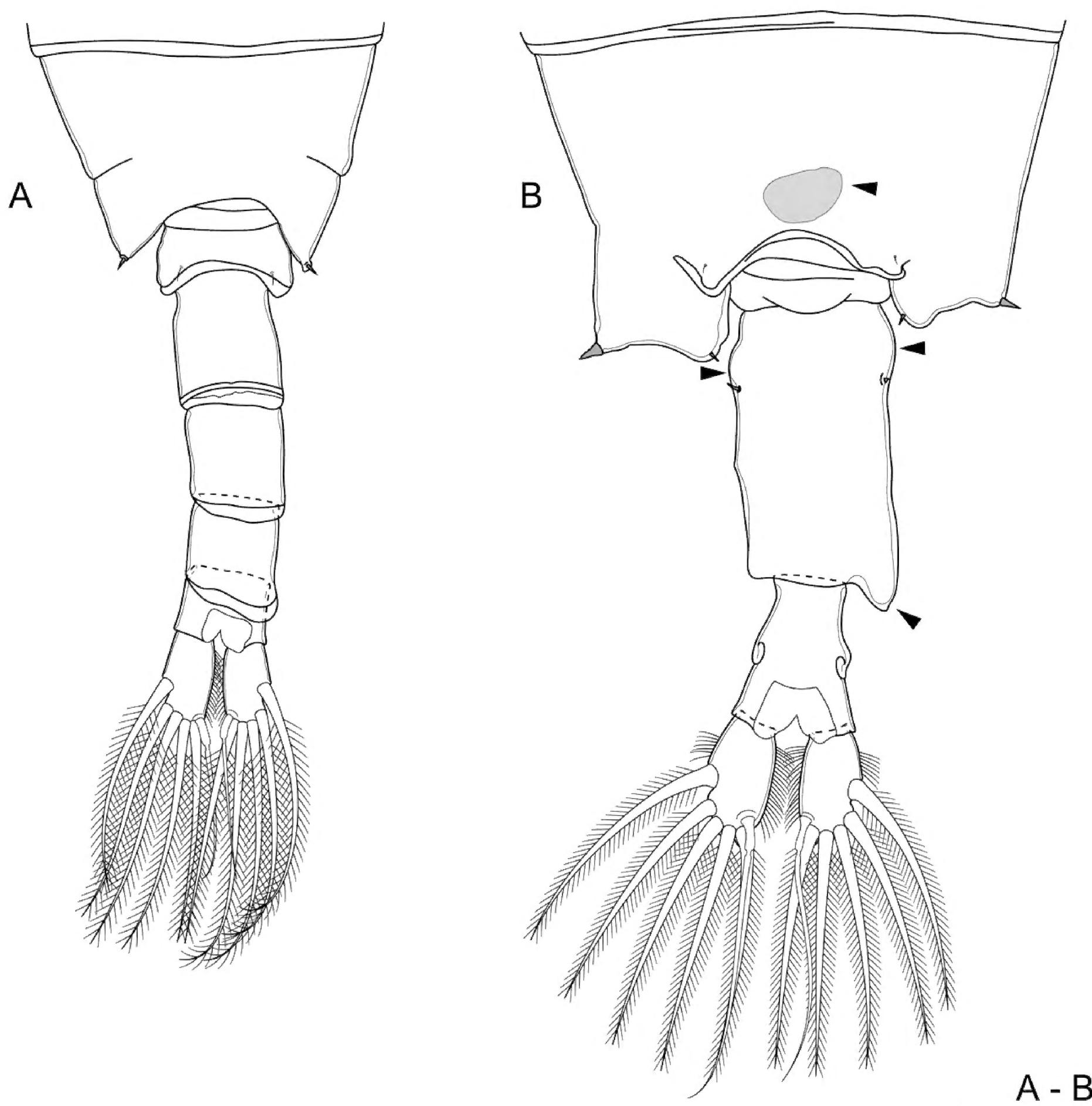


Figure 2. *Tropodiaptomus pedecrassum* sp. nov., male holotype. **A.** Pediger 5 and urosome, dorsal view; female allotype; **B.** Pediger 5 and urosome, dorsal view (arrowhead indicates anterior part swollen in both sides and triangular-like lobe, and grey ellipse indicates dorsal prominence). Scale bar: 100 µm.

plumose setae and the distal with eight plumose setae, and basal exite with one bipinnate spines. Exopod 1-segmented with six plumose setae, one longitudinal row of setules on inner margin of segment. Endopod 1-segmented with four plumose setae.

Maxilla (Fig. 4D). Proximal praecoxal endite with four setae, distal praecoxal endite with three setae. Proximal and distal coxal endites with three setae each. Allobasis protruding into endite with five setae. Endopod reduced to two segments, enp-1 with two setae and enp-2 with three setae.

Maxilliped (Fig. 4E). Praecoxal endite with one seta. Coxal endites with 2, 3, and 3 setae, respectively. Distal

corner of coxa produced into rounded lobe with spinules on inner margin. Basis with three setae on distal third, and one row of setules and one row of spinules on inner margin. Endopod 6-segmented; with 2, 3, 2, 2, 1+1, and 4 setae, respectively.

P1–P4 (Fig. 5A–D). Biramous. Intercoxal sclerite naked. Coxa with one inner seta. Basis without seta except P4 with one seta on outer distal margin. P1 with 3-segmented exopod and 2-segmented endopod, P2–P4 with 3-segmented exopod and endopod, endopod reaching proximal of exp-3. **P1** (Fig. 5A). Exp-1 with one longitudinal row of inner setules. Exp-2 and exp-3 with one longitudinal row of outer setules. Enp-2 with one row of spinules close

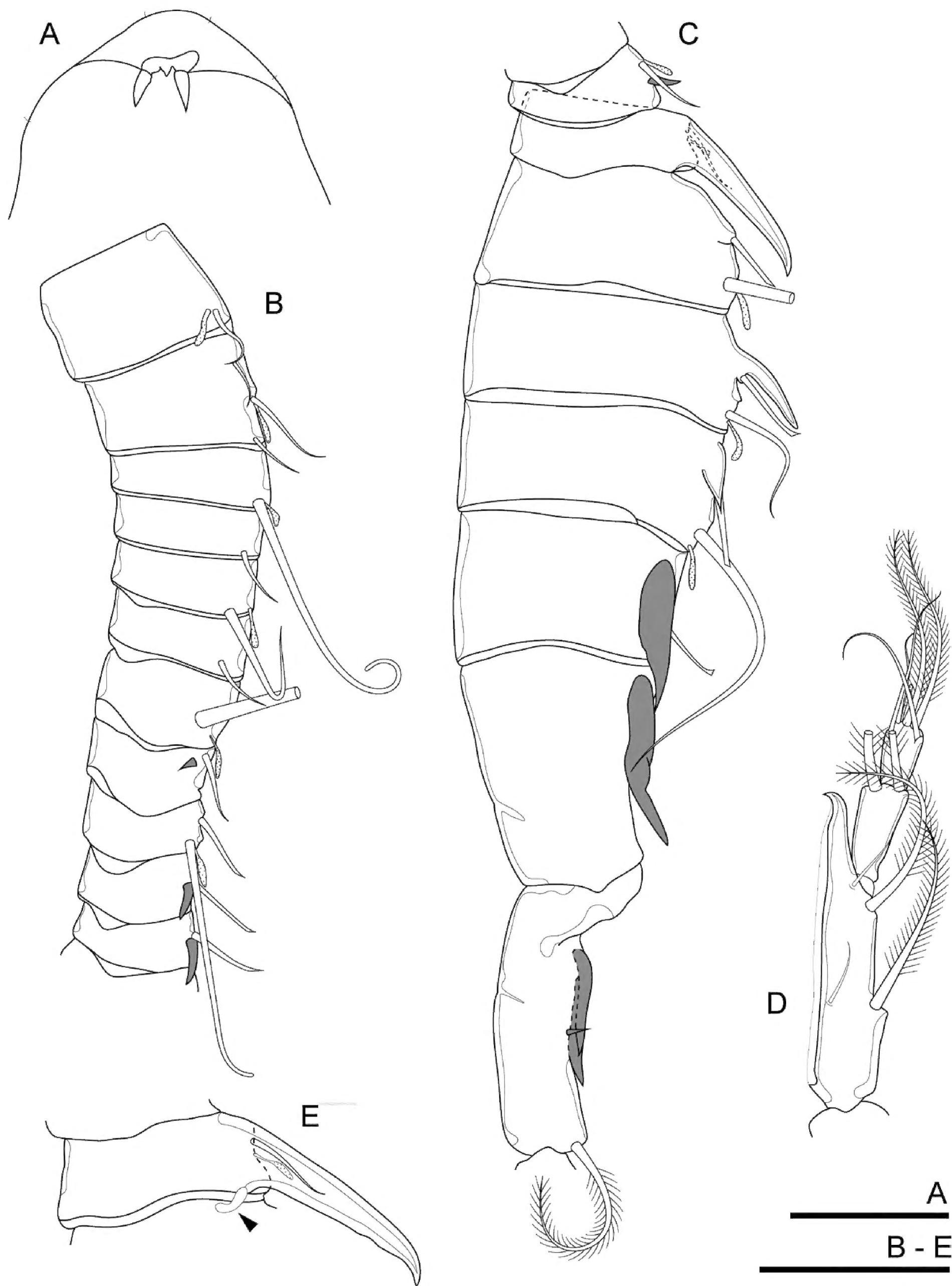


Figure 3. *Tropodiaptomus pedecrassum* sp. nov., male holotype. **A.** Rostrum; **B.** Segment 1–11 of right antennule; **C.** Segment 12–19 of right antennule (gray color indicate spine); **D.** Segment 20–22 of right antennule; **E.** Segment 13 of right antennule of the adult male specimen from the same population of holotype (arrowhead indicates hyaline knob). Scale bars: 100 µm.

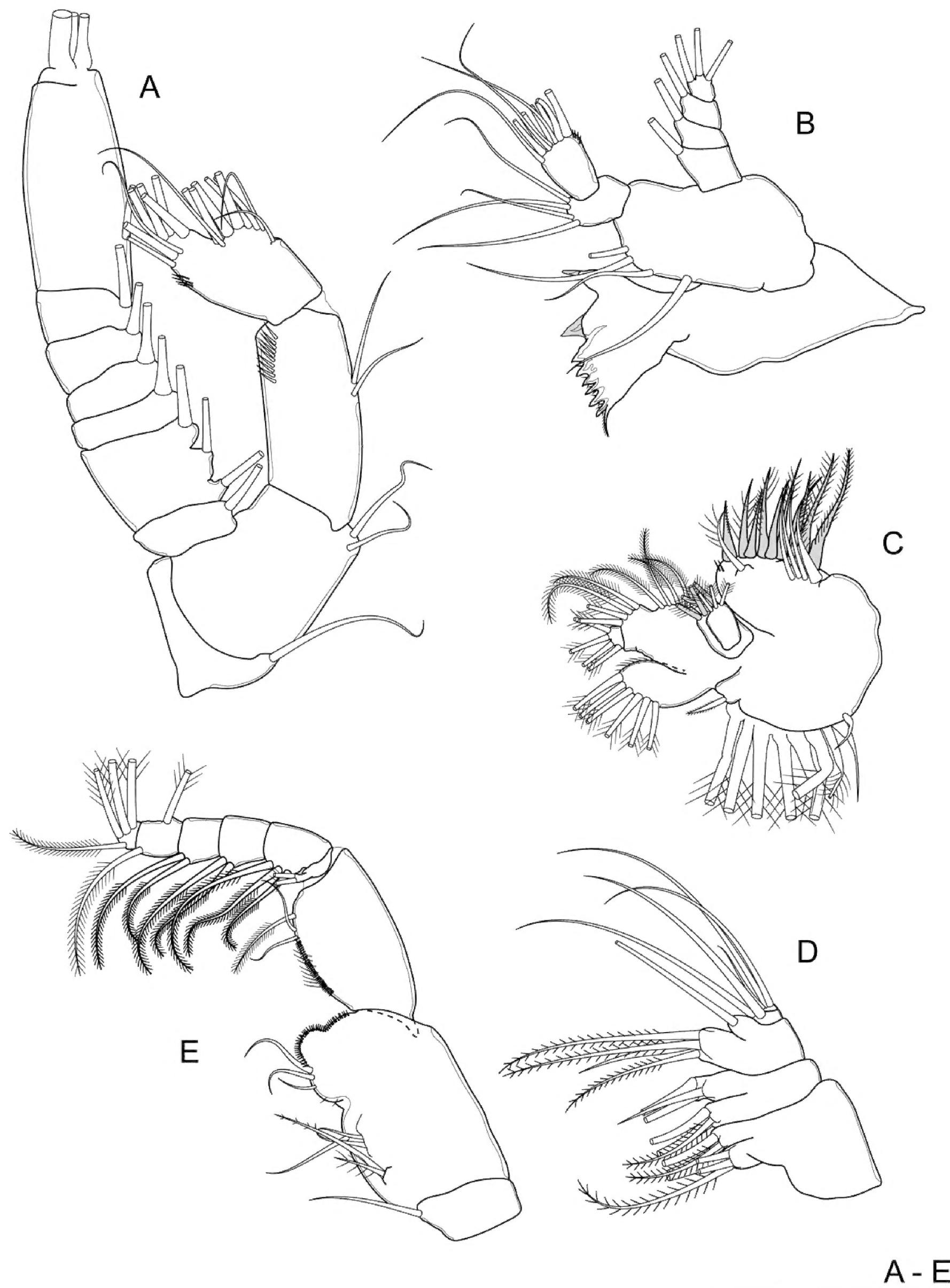


Figure 4. *Tropodiaptomus pedecrassum* sp. nov., male holotype. **A.** Antenna; **B.** Mandible; **C.** Maxillule; **D.** Maxilla; **E.** Maxilliped. Scale bar: 100 μ m.

to distal end. **P2–P4** (Fig. 5B–D). Exp-1 with one longitudinal row of inner setules. Exp-2 and exp-3 with one longitudinal row of outer and inner setules. Enp-1 with one longitudinal row of outer setules in P2 and P4. Enp-2

with one longitudinal row of outer setules in P2–P4, enp-2 of P2 with Schmeil's organ. Enp-3 with one longitudinal row of outer setules in P2, one longitudinal row of outer and inner setules in P4. Exp-3 and enp-3 with one and two

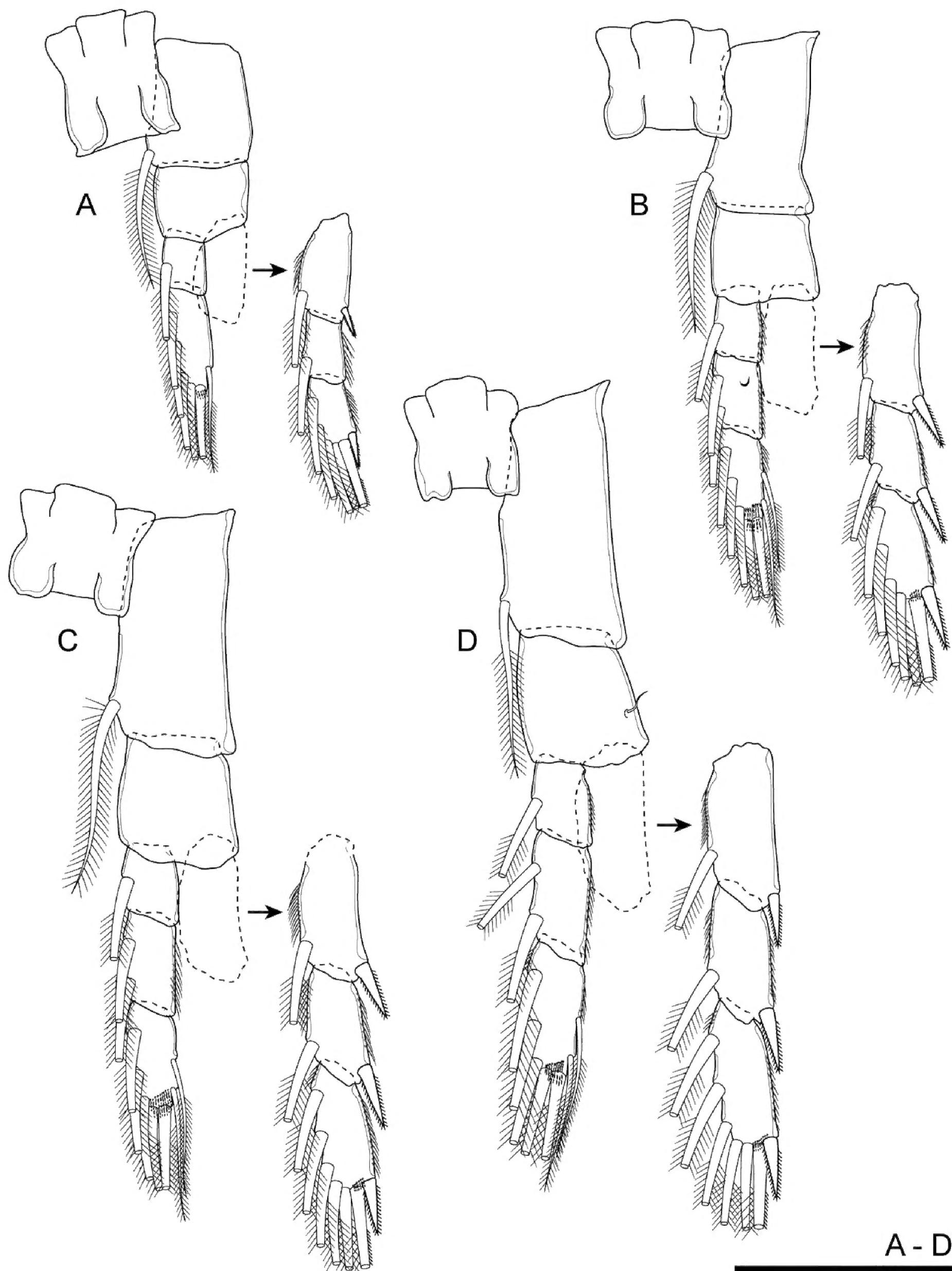


Figure 5. *Tropodiaptomus pedecrassum* sp. nov., male holotype. **A.** P1; **B.** P2; **C.** P3; **D.** P4. Scale bar: 100 μ m.

rows of outer spinules close to distal end in P2–P4, respectively. Armature formula of P1–P4 as in Table 1.

P5 (Figs 6A, 8A–D). Asymmetrical. **Left leg** (Figs 6A, 8A–D), reaching slightly beyond proximal margin of exp-

2 of right P5. Coxa as long as wide, with spine inserted on outer lobe. Basis cylindrical, about 1.4 times as long as wide, with one distal outer smooth seta. Exopod flattened, about 1.7 times as long as wide, inner margin one lobe, the

serration gradually decreases in size from the proximal to distal end (Fig. 8C, arrowhead). Apex of exopod with usual ‘finger-and-thumb’ combination, ‘finger’ slim, and set with radiant, hair-like ‘thumb’ sphere, ventral surface of exopod with 2 hairy pads. Endopod 2-segmented with incomplete separation of segments (Fig. 8D, arrowhead), conical, reaching beyond middle of exp-1, rounded distally with two parallel rows of setules. **Right leg** (Figs 6A, 8A, B), coxa as long as wide, with spine inserted on outer lobe. Basis cylindrical, about 1.3 times as long as wide, four structures occurring on dorsal surface: (i) one round process on proximal third close to inner margin, (ii) one triangular process in middle close to inner margin, (iii) one longitudinal hyaline lamella inserted near inner margin, and (iv) one distal outer smooth seta. Exopod 2-segmented. Exp-1 small, about 0.6 times as long as wide, with triangular lobe on inner margin, distal outer corner produced into acute spinous process, nearly as long as its segment. Exp-2 rhomboid, about 2.7 times as long as wide, dorsal surface with one semicircular hyaline knob on proximal outer margin, one semicircular hyaline lamella on distal inner margin, and one triangular process in middle of segment (Figs 6A, 8A, B (arrowhead)), and distal end of segment with one round hyaline prominence inserted between end claw and lateral spine (Fig. 8A, B (arrowhead)). Lateral spine nearly straight, acutely pointed, about as long as exp-2, inserted on distal corner of exp-2, with spinules on its inner margin. End claw curved and gradually tapering to acuminate tip, about 2.6 times as long as exp-2, inner margin with spinules distally. Endopod 1-segmented, conical, reaching distal end of exp-1, distal end with two rows of setules.

Description of the adult female. Body (Figs 1E, 2B, 6B, 8E, F). Total body length about 1,626 µm (1.6 mm) (measured from anterior margin of rostrum to posterior margin of caudal rami). Prosome length about 2.6 times as long as urosome (including caudal rami). Fourth and fifth pedigers completely fused, fusion being indicated by indentation on each side (Figs 1E, 2B). Fifth pediger produced into asymmetrical posterolateral wings (right wing shorter than left wing), each distal end with posterior spine, and each wing with one dorsal spine on inner margin (right spine smaller than left spine) (Figs 1E, 2B). Dorsal surface of fifth pediger with prominence (Figs 2B (grey ellipse), 8E (arrowhead)). Urosome 2-segmented. Genital double-somite asymmetrical, about 1.8 times as long as wide, both sides of anterior part swollen (Fig. 2B (arrowhead)), with two unequal dorsolateral spines (right spine smaller than left spine) in anterior third (Figs 1E,

2B, 8F). Right distal corner of genital double-somite one triangular-like lobe (Figs 2B (arrowhead), 8F (arrowhead)). Genital area on ventral surface shows opercular pad protecting gonopores, characterised by rectangular and semicircular expansions (Fig. 6B). Anal somite about 1.6 times as long as wide (Figs 1E, 2B, 8F). Caudal rami parallel (Figs 1E, 2B, 8F), symmetrical, about 1.9 times as long as wide, with setules on outer and inner margins (Figs 1E, 2B, 8F (arrowhead)). Each ramus with six setae.

A1, A2, mandible, maxillule, maxilla, maxilliped, P1–P4 and rostrum (not shown) same as male.

P5 (Figs 6C, 8G–I). Symmetrical. Coxal spine on posterior lobe on caudal surface. Basis with one smooth outer seta on distolateral margin. Exopod 3-segmented. Exp-1 cylindrical, length about 2.2 times as long as wide. Exp-2 tapering into long claw, each side with one row of spinules starting in middle of segment. Exp-3 fused with exp-2 (Figs 6C (arrowhead), 8I (arrowhead)), with two unequal spines; inner spine about 3.6 times as long as outer spine, and with short spine laterally. Endopod 1-segmented, cylindrical, length about 0.6 times as long as exp-1, two unequal strong smooth spiniform setae distally (Figs 6C, 8G, H (arrowhead)), outer seta longer than inner seta, two parallel rows of spinules on distal end.

Variability. Morphological variability has been observed in: (i) the total body length (except of caudal setae) which ranged from 1,145–1,380 µm (mean 1,283 µm, n=6) in the adult males and 1,520–1,626 µm (mean 1,575 µm, n=5) in the adult females; (ii) the length of the spinous process on antepenultimate segment of the adult male right A1 is 3/4 to equal of segment 21 (Fig. 7A–G; see Table 2 in Saetang et al. 2022); (iii) segment 13 of the adult male right antennule with one hyaline knob (Fig. 3E (arrowhead)); and (iv) the number of setae on segment 13 of the adult male left A1 has one seta (n=10) or two setae (n=2) (Fig. 1D (arrowhead); see Table 2 in Saetang et al. 2022)

Etymology. The specific name ‘*pedecrassum*’ is derived from the chubby shape of the adult male P5 that is clearly different from the more rectangular shape in other species of the genus.

Co-occurring species. In our samples, the new taxon co-occurred with one other copepod species, *Mongolodiaptomus botulifer* (Kiefer, 1974).

Distribution and ecology. *Tropodiaptomus pedecrassum* sp. nov. was found only in its type locality so far. It was recorded in two out of 471 samples collected from 206 freshwater habitats throughout Thailand between September 2017 and July 2019. Water temperature 19.6 °C, conductivity 620 µs cm⁻¹, salinity

Table 1. Armature formula of P1–P4 in *T. pedecrassum* sp. nov. and *T. longiprocessus* sp. nov. (Arabic numerals representing setae and Roman numerals representing spine from outer-inner or outer-apical-inner margins).

Swimming legs	Coxa	Basis	Exp			Enp		
			1	2	3	1	2	3
P1	0–1	0–0	I–1	0–1	I,3,2	0–1	1,2,3	–
P2	0–1	0–0	I–1	I–1	I,3,3	0–1	0–2	2,2,3
P3	0–1	0–0	I–1	I–1	I,3,3	0–1	0–2	2,2,3
P4	0–1	1–0	I–1	I–1	I,3,3	0–1	0–2	2,2,3

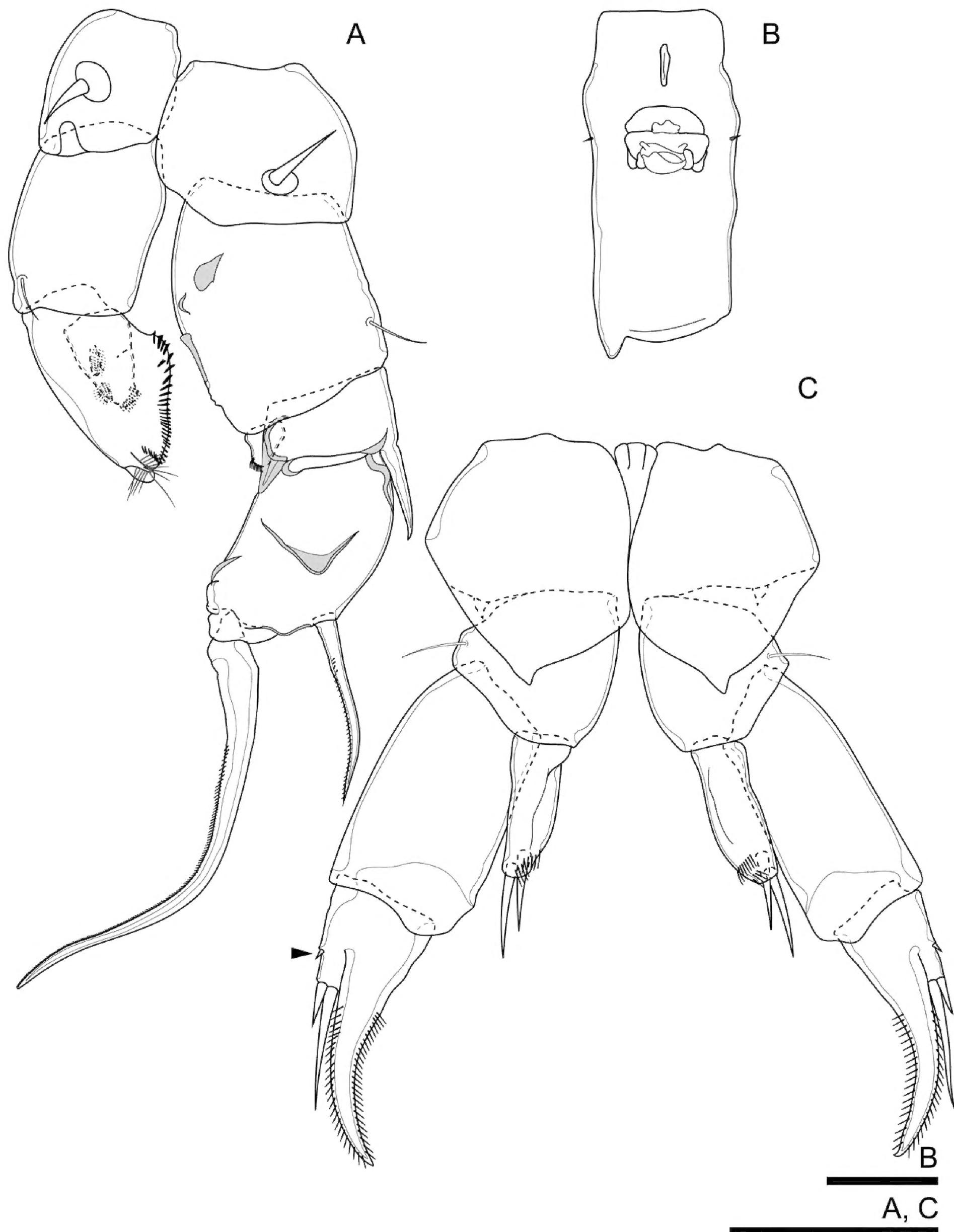


Figure 6. *Tropodiaptomus pedecrassum* sp. nov., male holotype. **A.** P5, dorsal view; *Tropodiaptomus pedecrassum* sp. nov., female allotype; **B.** genital double-somite, ventral view; **C.** P5, dorsal view (arrowhead indicates exp-3). Scale bars: 100 µm.

0.3 ppt, total dissolved solids 450 mg L⁻¹, dissolved oxygen 3.3 mg L⁻¹, pH 7.1, and water depth 30–40 cm, substrate with mud.

Differential diagnosis. *Tropodiaptomus pedecrassum* sp. nov. is confirmed to belong to the genus *Tropodiaptomus* based on the combination of characteristics men-

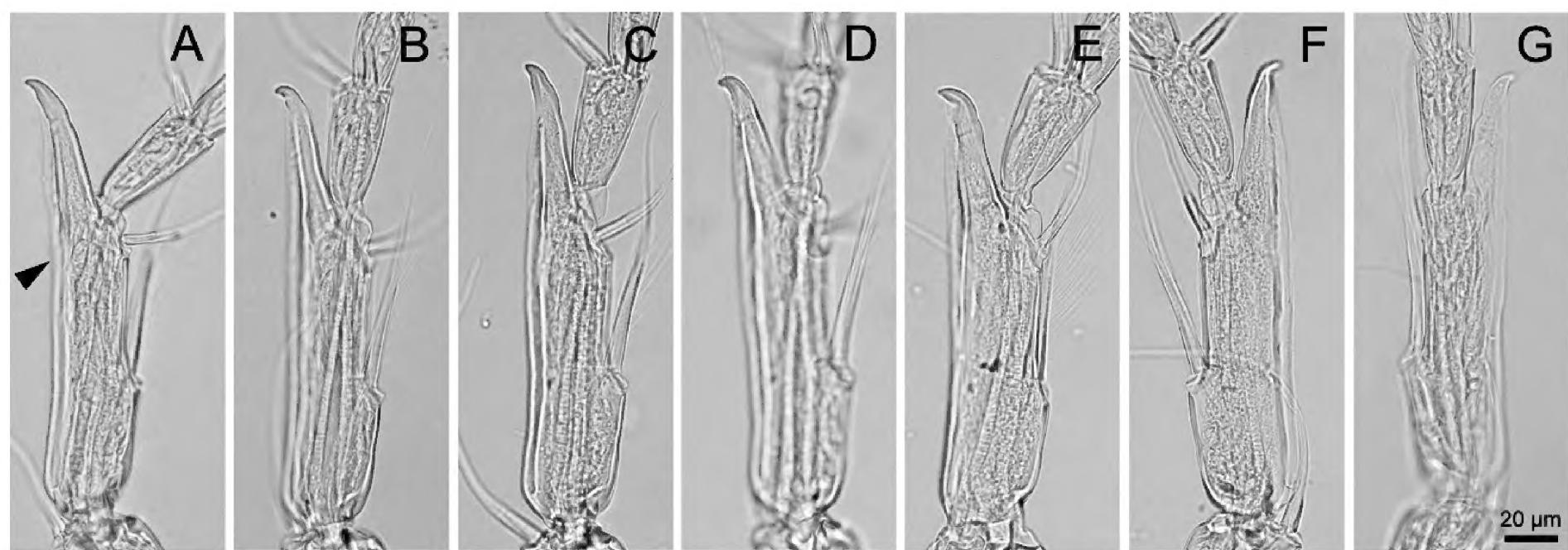


Figure 7. *Tropodiaptomus pedecrassum* sp. nov.; morphological variation of antepenultimate segment of the male right antennule (arrowhead indicates longitudinal hyaline membrane).

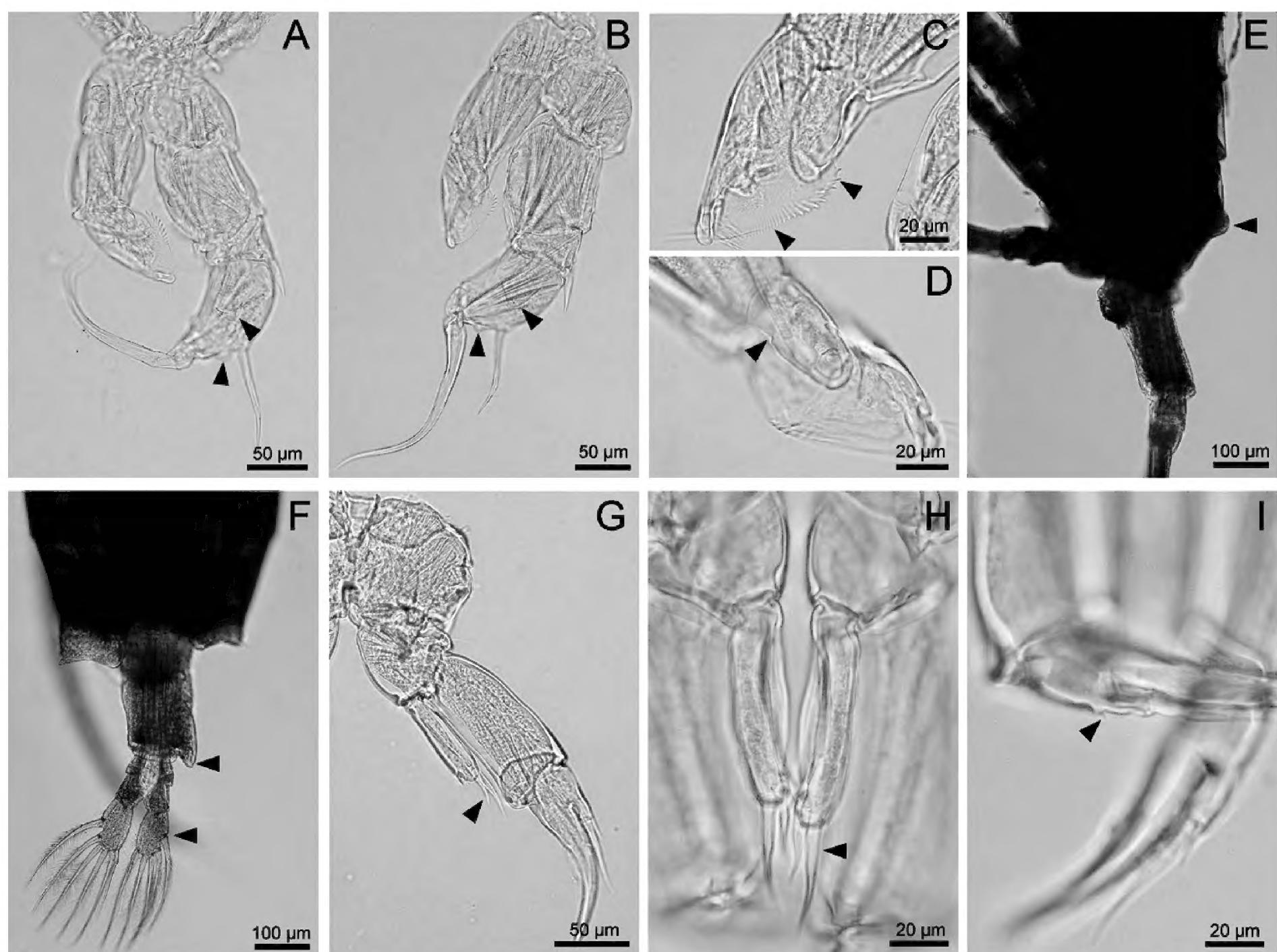


Figure 8. *Tropodiaptomus pedecrassum* sp. nov., male. **A, B.** P5, dorsal view; **c, d.** Exopodal and endopodal segment of left P5; female. **E.** Pediger 5 and urosome, lateral view; **F.** Pediger 5 and urosome, dorsal view; **g.** P5, dorsal view; **h.** Endopod of P5; **i.** Exp-2 and exp-3 of P5. Arrowheads show structures discussed in the description.

tioned by Lai and Fernando (1979a, 1979b), Sanoamuang (2002), Saetang et al. (2020): (i) the process on the antepenultimate segment of the adult male right antennule is always smooth; (ii) the exopod of the adult male left P5 fused into a single flattened piece and its inner margin is denticulate or serrate; (iii) the inner margin on the basis of the adult male right P5 has a hyaline lobe; (iv) the

urosome of the adult female comprises two somites; and (v) the endopod segment of the adult female P5 has slender setae at the distal end.

This species differs from the congeneric species by the following characters: (i) antepenultimate segment of the male right antennule with straight spinous process reaching 3/4 or equal of next segment; (ii) inner margin of

Table 2. Comparison of four morphologically similar species of *Tropodiaptomus* (after Kiefer 1930; 1982 and this study).

Morphological characters	<i>T. pedecrassum</i> sp. nov.	<i>T. longiprocessus</i> sp. nov.	<i>T. hebereri</i>	<i>T. lanaonus</i>
The adult male				
Body length, except caudal setae (μm)	1,145–1,380	1,490–1,545	1,350–1,400	1,120–1,150
Right A1: segment-16 with spinous process	Yes	Yes	No	No
Right A1: relative length of the spinous process of segment 20	3/4 of or equal to segment 21	Longer than segment 21	1/2 of segment 21	Longer than segment 21
Left A1: number of setae on segment-13	1 or 2	1	1	1
Right P5: basis, supplementary process and surface structures	One round process, one semicircular process and one longitudinal hyaline lamella	One triangular process and one longitudinal hyaline lamella	One triangular process, one semicircular process and one semicircular hyaline lamella	One triangular process and one longitudinal hyaline lamella
Right P5: exp-1, length of spinous process on outer distal margin/segment length	Equal	Shorter	Shorter	Shorter
Right P5: exp-2, shape	Rhomboid	Rectangular	Trapezoidal	Rectangular
Right P5: exp-2, supplementary process	Triangular-shape process, large	Large and long process	Triangular process	Semicircular process
Right P5: lateral spine of the exp-2	Short	Long	Short	Long
Right P5: exp-2, one round hyaline prominence inserted between end claw and lateral spine	Present	Absent	Present	Absent
Right P5: length of exp-1 vs exp-2 length	Shorter	Longer	Longer	Longer
Left P5: exp, inner margin	1 lobe, the serration gradually decreasing in size from proximal to distal end	2 lobes, shallow, uniform serration	1 lobe, the serration of the proximal part larger than the distal part	2 lobes, shallow, uniform serration
Left P5: exp (number of segment)	2 (yet incompletely separated)	2	1	2
Left P5: length of finger vs thumb length	Equal	Equal	3/4	Equal
The adult female				
Body length, except caudal setae (μm)	1,520–1,626	1,570–1,700	1,500–1,600	1,200–1,250
Fifth pediger: lateral wings	Asymmetrical	Asymmetrical	Asymmetrical	Symmetrical
Fifth pediger: dorsal prominence	Present	Absent	Present	Absent
Genital double-somite	Asymmetrical, dilate on proximal both sides	Asymmetrical, dilate on proximal right side	Asymmetrical, dilate on proximal both sides	Symmetrical
Genital double-somite: outer distal corner with lobe	Triangular lobe	Absent	Large round lobe	Small round lobe
Caudal rami: outer margin with setules	Present	Present	Absent	Present
P5: exp-3 fused with exp-2	Yes	Yes	No	No data
P5: end claw, outer margin with setules	Present	Present	Present	No data
P5: exp, elements on distal end	Spiniform smooth setae	Spiniform smooth setae	Slender smooth seta	Slender smooth seta

exopodal segment of the male left P5 with single lobe and the serration gradually decreases in size from the proximal to distal end; (iii) basis of the male right P5 with two processes and one longitudinal hyaline lamella; (iv) exp-1 of the male right P5 with triangular lobe on inner margin, distal outer corner produced into acute spinous process, length about as long as its segment; and (v) exp-2 of the male right P5 with rhomboid shape, dorsal surface with one semicircular hyaline knob on proximal outer margin, one semicircular hyaline lamella on distal inner margin, and one triangular process in middle of segment.

According to the identification key given by Saetang et al. (2020), *Tropodiaptomus pedecrassum* sp. nov. is most similar to *T. hebereri*. However, it distinctly differs from *T. hebereri* in the following characters: (i) inner margin of the basis of the adult male right P5 has one round process, one semicircular process, and one longitudinal hyaline lamella; (ii) length of spinous process of the outer corner of exp-1 of the adult male right P5 is as long as exp-1; (iii) inner margin of the exopod of the adult male left P5 has one lobe, and the serration gradually decreases in size from the proximal to the distal end; (iv) dorsal surface of exp-2 of the adult male right

P5 has one semicircular hyaline knob, one semi-circular hyaline lamella, and one triangular process; and (v) caudal rami of the adult female have outer and inner setules (Table 2).

Tropodiaptomus longiprocessus sp. nov.

<https://zoobank.org/F33BE672-BC19-4644-8477-D4C6F817D397>

Type locality. Nong Ping swamp (NP2), Thong Phaphum district, Kanchanaburi province, western Thailand (14°38'49.1"N, 98°33'48.8"E). Temporary habitat without macrophytes.

Other localities. Nong Ping swamp (NP3) Thong Phaphum District, Kanchanaburi Province, Western Thailand (14°39'00.4"N, 98°34'33.7"E). Temporary habitat without macrophytes.

Material examined. **Holotype.** Adult male, dissected and mounted onto one slide, Thong Phaphum District, Kanchanaburi province, western Thailand (14°38'49.1"N, 98°33'48.8"E), 22 June 2019, Thanida Saetang and Supiyanit Maiphae; PSUZC-PK2009-01. **Allotype.** One adult female, collected with holotype; PSUZC-PK2009-02.

Paratype. One adult male, collected with holotype; PSU-ZC-PK2009-03.

Description of the adult male. **Body** (Fig. 9A, B). Total body length about 1,500 μm (measured from anterior margin of rostrum to posterior margin of caudal rami). Prosome length about 2.4 times as long as urosome (including caudal rami). Fourth and fifth pedigers separated by distinct septum. Fifth pediger produced into small symmetrical posterolateral wings, each distal end with spine. Urosome 5-segmented. Genital somite with dorso-lateral sensillum on right side, fourth somite with expanded right corner. Anal somite with deep cleft, length as long as wide. Caudal rami parallel, symmetrical, length about 1.9 times as long as wide, with setules on inner margin. Each ramus with six setae.

Rostrum (Fig. 9C). Two rostral elements on anterior margin with suture in the middle.

A1 (Figs 10A–C, 11A, B, 16H). Asymmetrical. **Left A1** non-geniculate, 25-segmented, reaching beyond the end of caudal rami. Armature formula of each segment as follows: 1+ae, 3+ae, 1+ae, 1, 1+ae, 1, 1+ae, 1+s, 2+ae, 1, 1, 1+ae+s, 1, 1+ae, 1, 1+ae, 1, 1, 1+ae, 1, 1, 2, 2, 2, 5+ae. **Right A1** transformed and geniculate, 22-segmented. Strongly dilated between segment 13 and segment 18. Spinous process on segment 20 (antepenultimate) straight and bent at distal end, reaching longer than next segment, and with longitudinal hyaline membrane on outer margin (Fig. 10D). Armature formula of each segment as follows: 1+ae, 3+ae, 1+ae, 1, 1+ae, 1, 1+ae, 1+s, 2+ae, 1+sp, 1+sp, 1+ae+s, 1+ae+sp, 2+ae, 2+ae+sp, 2+ae+sp, 1+s, 1+3s, 4+sp, 2, 5+ae.

A2 (Fig. 9D). Coxa with one inner seta on distal corner. Basis with two inner setae on distal corner. Exopod 7-segmented, exp-1–6 with 1, 3, 1, 1, 1, and 1 inner setae, respectively, and exp-7 with one inner and three apical setae. Endopod 2-segmented, enp-1 with two inner setae and one longitudinal row of outer spinules, enp-2 with nine inner and seven apical setae; and one group of outer spinules.

Mandible (Fig. 12A). Coxa with eight strongly chitinised teeth and one seta on gnathobase. Basis with four inner setae. Exopod 4-segmented with 1, 1, 1, and 3 setae, respectively. Endopod 2-segmented, enp-1 with four inner setae, enp-2 with nine apical setae and three horizontal rows of outer spinules.

Maxillule (Fig. 12B–E). Precoxal arthrite with three plumose setae and twelve bipinnate spines. Coxal endite with four plumose setae, and coxal epipodite with seven plumose setae and two bipinnate spines. Basis with two endites; the proximal with four plumose setae and the distal with eight plumose setae, and basal exite with one bipinnate spine. Exopod 1-segmented with six plumose setae, one longitudinal row of inner setules. Endopod 1-segmented with four plumose setae and one horizontal row of outer setules.

Maxilla (Fig. 12F). Proximal praecoxal endite with six setae, distal praecoxal endite with three setae. Proximal and distal coxal endites with three setae each. Allobasis

protruding into endite with four setae. Endopod reduced to two segments, each with three setae.

Maxilliped (Fig. 12G). Praecoxa completely fused to coxa; endites with 1, 2, 3, and 3 setae, respectively. Distal corner of coxa produced into rounded lobe with spinules on inner margin. Basis with three setae in distal third, one row of setules and one row of spinules in proximal half. Endopod 6-segmented; with 2, 3, 2, 2, 1+1, and 4 setae, respectively.

P1–P4 (Figs 13A–D, 16I). Biramous. Intercoxal sclerite naked. Coxa with one inner seta. Basis without seta except P4 with one seta on outer distal margin. P1 with 3-segmented exopod and 2-segmented endopod, P2–P4 with 3-segmented exopod and endopod, endopod reaching proximal of exp-3. P1 (Figs 13A, 16I). Basis with lateral setules close to outer margin. Exp-2, exp-3, enp-1 and enp-2 with one longitudinal row of outer setules. Exp-3 and enp-2 with one row of spinules close to distal end. P2–P4 (Fig. 13B–D). Exp-1 with one longitudinal row of inner setules. Exp-2 and exp-3 with one longitudinal row of outer and inner setules in P2 and P4, and one longitudinal row of inner setules in P3. Enp-1 with one longitudinal row of outer setules in P3 and P4. Enp-2 and enp-3 with one longitudinal row of outer setules in P2, one longitudinal row of outer and inner setules in P3 and P4, enp-2 of P2 with Schmeil's organ. Exp-3 and enp-3 with one and two rows of outer spinules close to distal end in P2–P4, respectively. Armature formula of P1–P4 as same as *Tropodiaptomus pedecrassum* sp. nov. (Table 1).

P5 (Figs 14A, E, 16A, E). Asymmetrical. **Left leg**, reaching beyond middle of exp-2 of right P5. Coxa as long as wide, with spine inserted on outer lobe. Basis cylindrical, about 1.9 times as long as wide, with one distal outer smooth seta. Exopod flattened, about 2.2 times as long as wide, inner margin two lobes with uniform serration (Figs 14A, 16A, E). Apex of exopod with usual 'finger-and-thumb' combination, 'finger' slim, and set with radiant, hair-like 'thumb' sphere, ventral surface of exopod with two hairy pads. Endopod 2-segmented (Fig. 16G), conical, reaching beyond middle of exp-1, rounded distally with two parallel rows of setules. **Right leg**, coxa as long as wide, with spine inserted on outer lobe. Basis cylindrical, about 1.7 times as long as wide, three structures occurring on dorsal surface: (i) one triangular process in proximal third close to inner margin, (ii) one longitudinal hyaline lamella inserted near inner margin in middle, and (iii) one laterodistal smooth seta. Exopod 2-segmented. Exp-1 small, about 0.5 times as long as wide, with triangular lobe on inner margin, laterodistal corner produced into acute spinous process. Exp-2 rectangular, about 1.5 times as long as wide, dorsal surface with two longitudinal hyaline lamellae in middle and distal inner margin and one large and long process in middle near outer margin. Lateral spine nearly straight, acutely pointed, about 1.3 times as long as exp-2, inserted in laterodistal corner of exp-2, with spinules on its inner margin. End claw curved and gradually tapering to acuminate tip, about 2.5 times

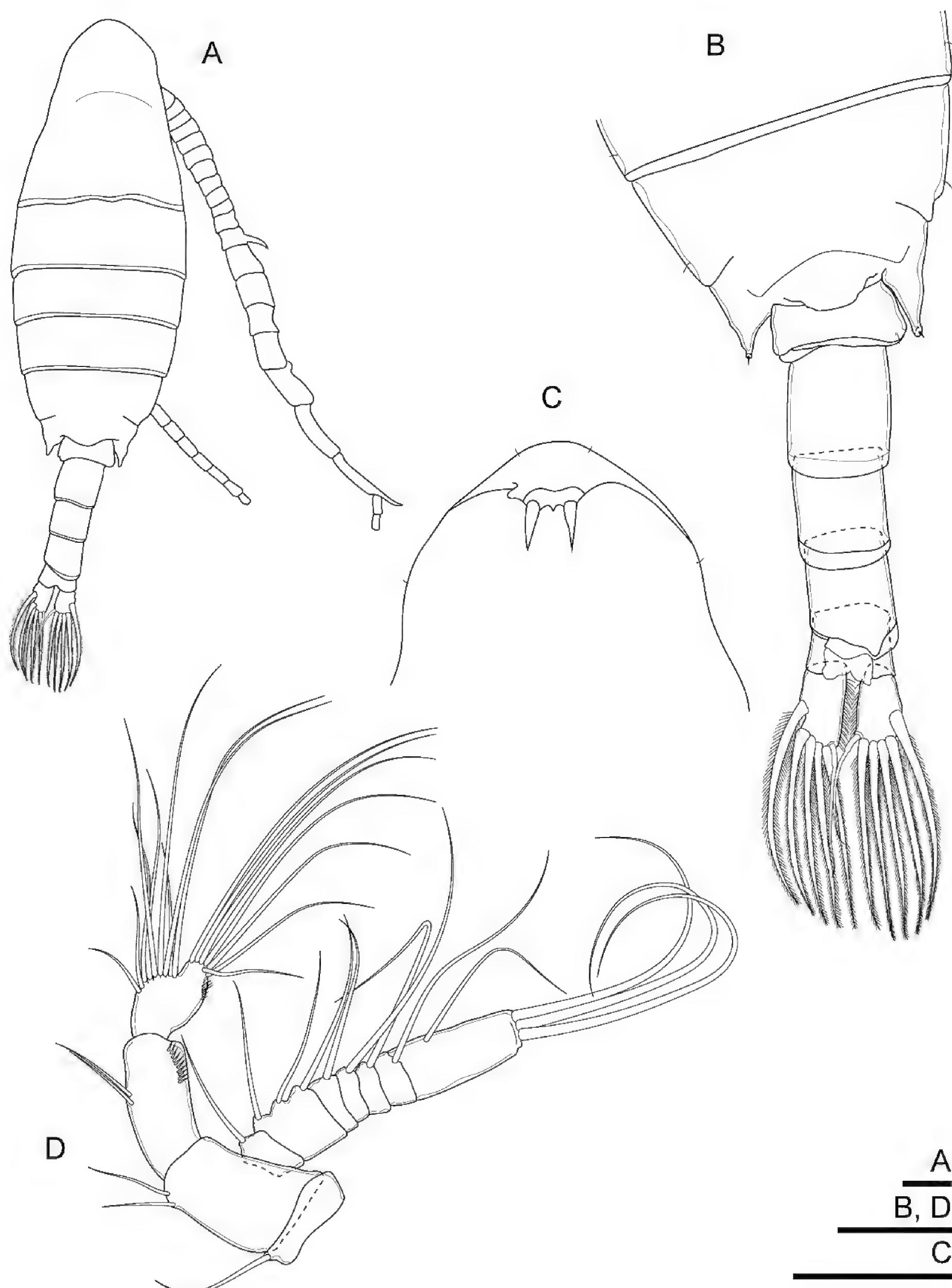


Figure 9. *Tropodiaptomus longiprocessus* sp. nov., male holotype. **A.** habitus, dorsal view; **B.** pediger 5 and urosome, dorsal view; **C.** rostrum; **D.** antenna. Scale bars: 100 μ m.

as long as exp-2, inner margin with spinules distally. Endopod 1-segmented, conical, reaching beyond proximal margin of exp-2, distal end with two rows of setules.

Description of the adult female. Body (Figs 15A–C, 16J, K). Total body length about 1,640 μ m (1.6 mm) (measured from anterior margin of rostrum to posterior

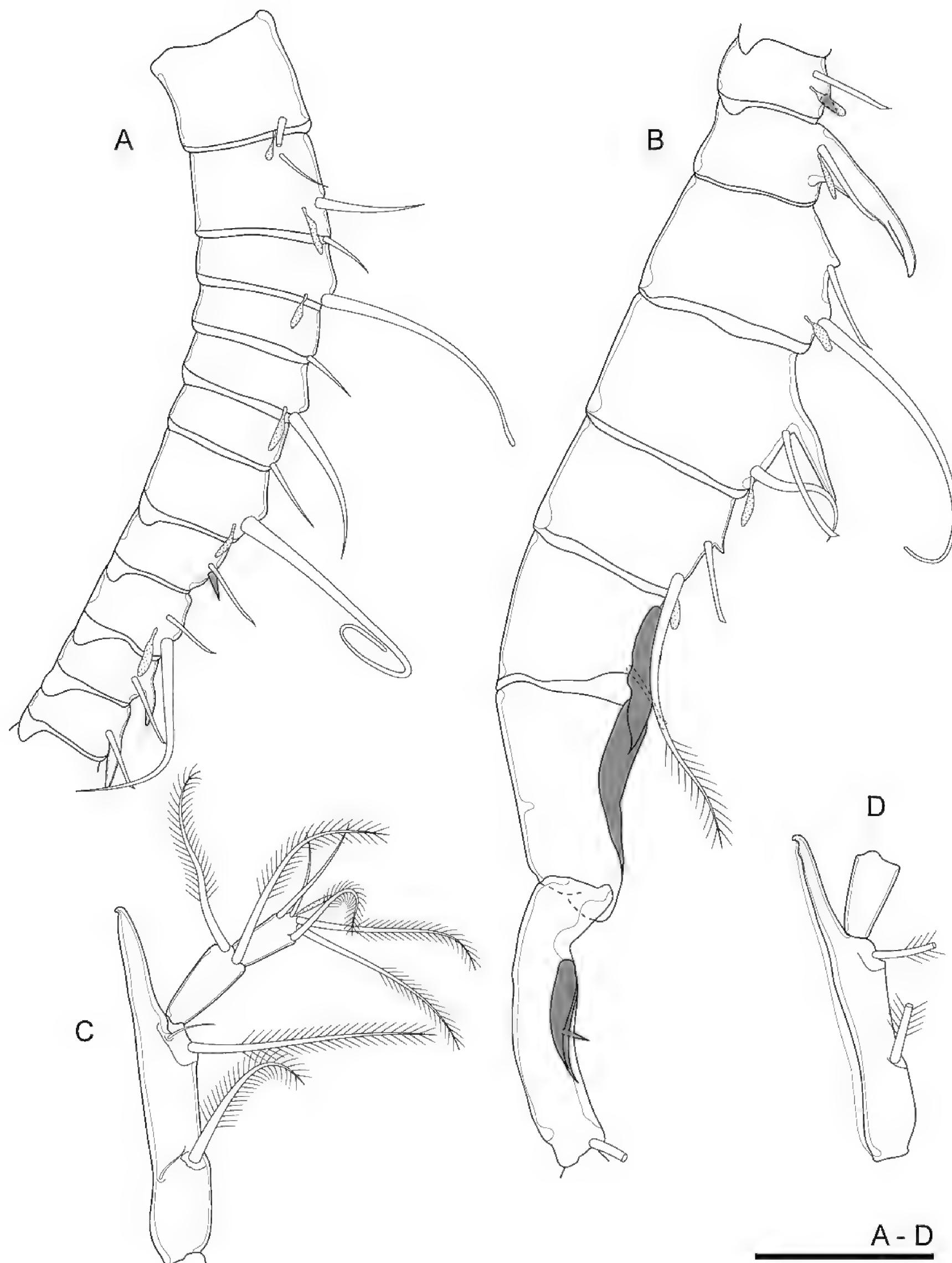


Figure 10. *Tropodiaptomus longiprocessus* sp. nov., male holotype. **A.** Segment 1–11 of right antennule; **B.** Segment 12–19 of right antennule (gray color indicates spine); **C.** Segment 20–22 of right antennule; **D.** Segment 20 and 21 of right antennule of the adult male specimen from the same population of holotype. Scale bar: 100 μ m.

margin of caudal rami). Prosome length about 3.1 times as long as urosome (including caudal rami). Fourth and fifth pedigers completely fused, fusion being indicated by indentation on each side. Fifth pediger produced into asymmetrical posterolateral wings (right wing shorter than left wing), each distal end with posterior spine, and

each wing with one dorsal spine on inner margin (right spine smaller than left spine) (Fig. 16K). Dorsal surface of fifth pediger without prominence (Fig. 16J). Urosome 2-segmented. Genital double-somite asymmetrical, about twice times as long as wide, right sides of anterior part swollen (Figs 15B (arrowhead), 16K), with two

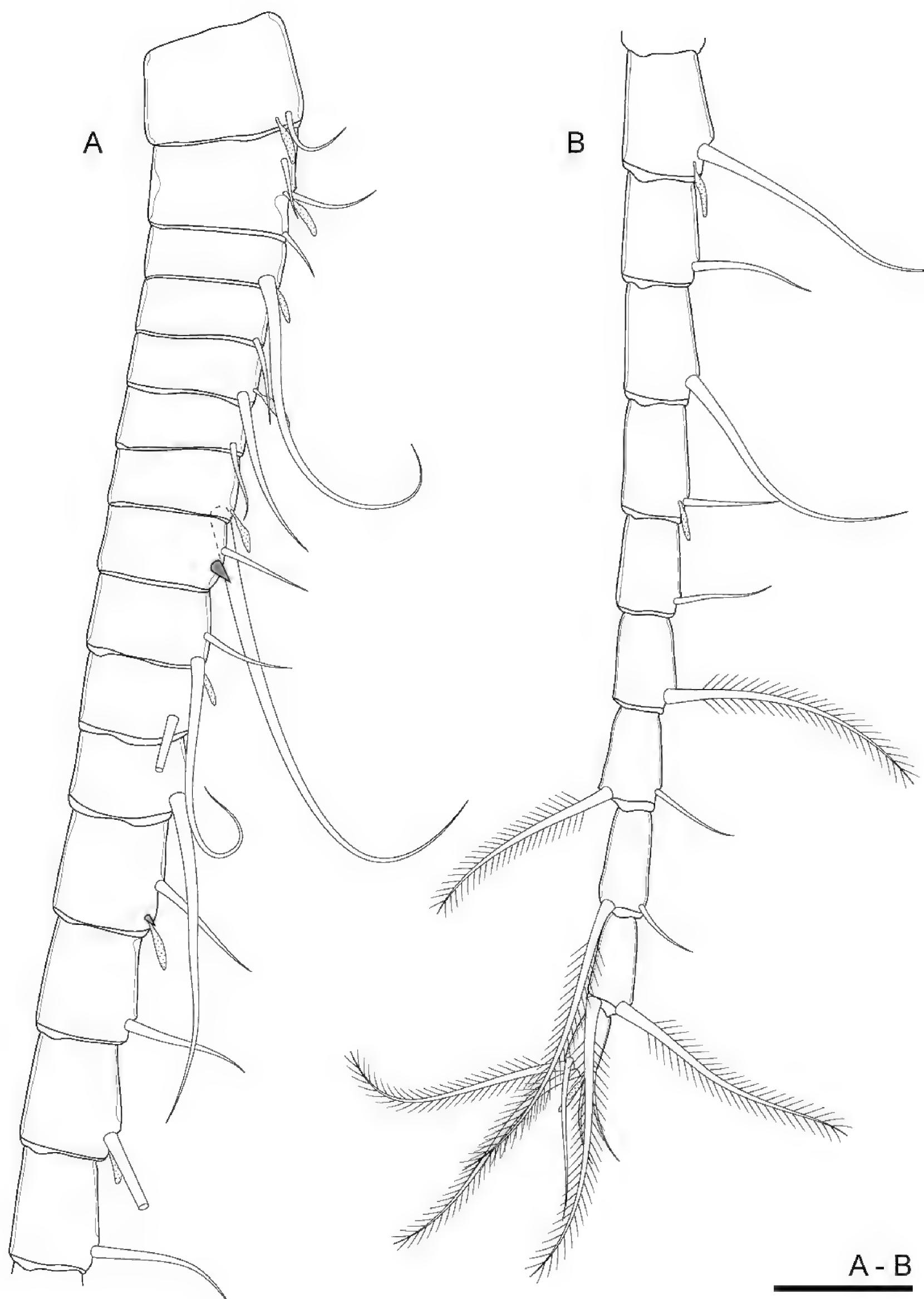


Figure 11. *Tropodiaptomus longiprocessus* sp. nov., male holotype. **A.** Left antennule, segment 1–15; **B.** Left antennule, segment 16–25 (gray color indicates spine). Scale bar: 100 μ m.

unequal dorsolateral spines (right spine smaller than left spine) in anterior third (Fig. 15B). Right distal corner of genital double-somite without lobe (Figs 15B, 16K (arrowhead)). Genital area on ventral surface shows opercular pad protecting gonopores, characterised by rectangular and semicircular expansions (Fig. 15C). Anal

somite about 1.2 times as long as wide. Caudal rami parallel, symmetrical, about 1.5 times as long as wide, with setules on outer and inner margins (Fig. 15B). Each ramus with six setae.

A1, A2, mandible, maxillule, maxilla, maxilliped, P1–P4 (not shown) and rostrum (Fig. 15D) same as male.

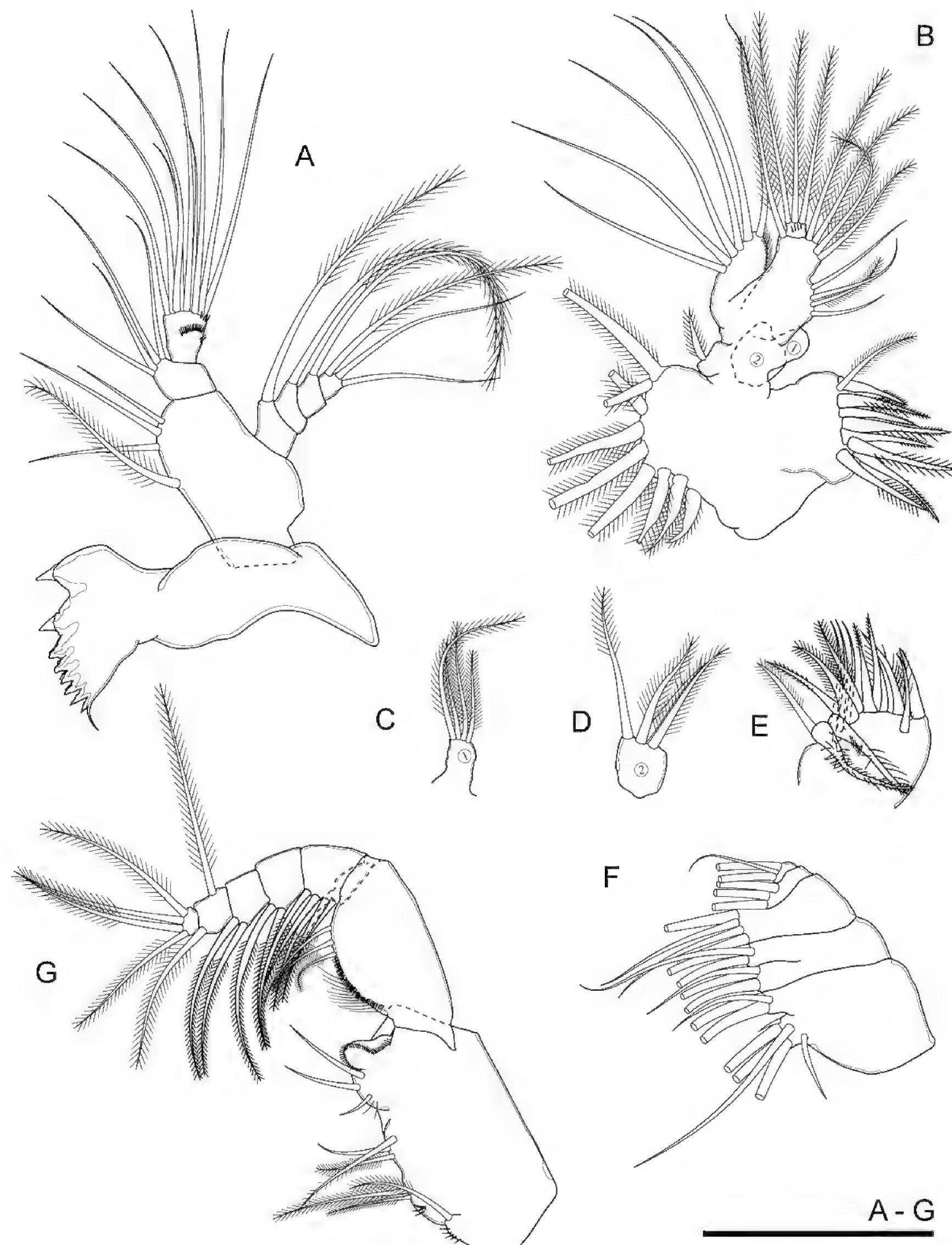


Figure 12. *Tropodiaptomus longiprocessus* sp. nov., male holotype. **A.** Mandible; **B.** Maxillule; **C–E.** Maxillule; **F.** Maxilla; **G.** Maxilliped. Scale bar: 100 μ m.

P5 (Figs 14F, 16L). Symmetrical. Coxa spine on posterior lobe on caudal surface. Basis with one smooth outer seta on distolateral margin. Exopod 3-segmented. Exp-1 cylindrical, length about 1.9 time as long as wide. Exp-2 tapering into long claw, each side with one row of spinules starting in middle of segment. Exp-3 fused with exp-2 (Figs 14F, 16L (arrowhead)), with two unequal spines, inner spine

about 4.9 times as long as outer spine, and with short spine laterally. Endopod 1-segmented, cylindrical, length about 0.7 time as long as exp-1, two strong smooth spiniform setae distally (Figs 14F, 16L (arrowhead)), outer seta longer than inner seta, two parallel rows of spinules on distal end.

Variability. Morphological variability has been observed in: (i) the total body length (except of cau-

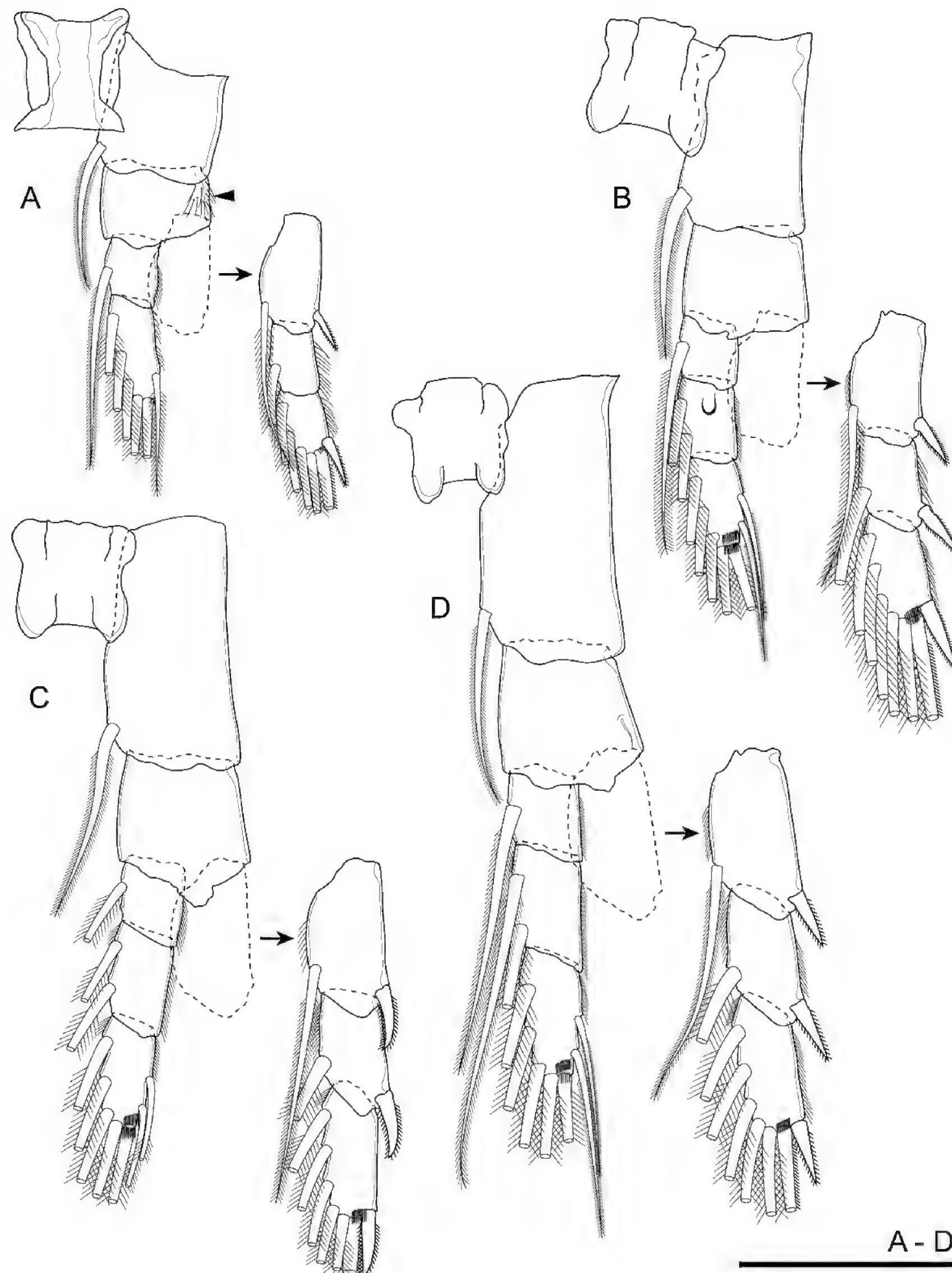


Figure 13. *Tropodiaptomus longiprocessus* sp. nov., male holotype. **A.** P1 (arrowhead indicates lateral setules close to outer margin); **B.** P2; **C.** P3; **D.** P4. Scale bar: 100 μ m.

dal setae) which ranged from 1,490–1,545 μ m (mean 1,510 μ m, $n = 4$) in the adult males and 1,570–1,700 μ m (mean 1,646 μ m, $n = 6$) in the adult females; and (ii) shape and length of large and long process on exp-2 of the adult male right P5 (Figs 14B–D, 16B–D). Moreover, it needs to be noted that the inner margin of exopod of the adult male left P5 appeared single lobe in

positions other than dorsal which can lead to misidentification (Fig. 16F).

Etymology. The specific name ‘*longiprocessus*’ is derived from the presence of a long process on exp-2 of the right P5 in the adult male.

Co-occurring species. There were no other diaptomid copepods in these samples.

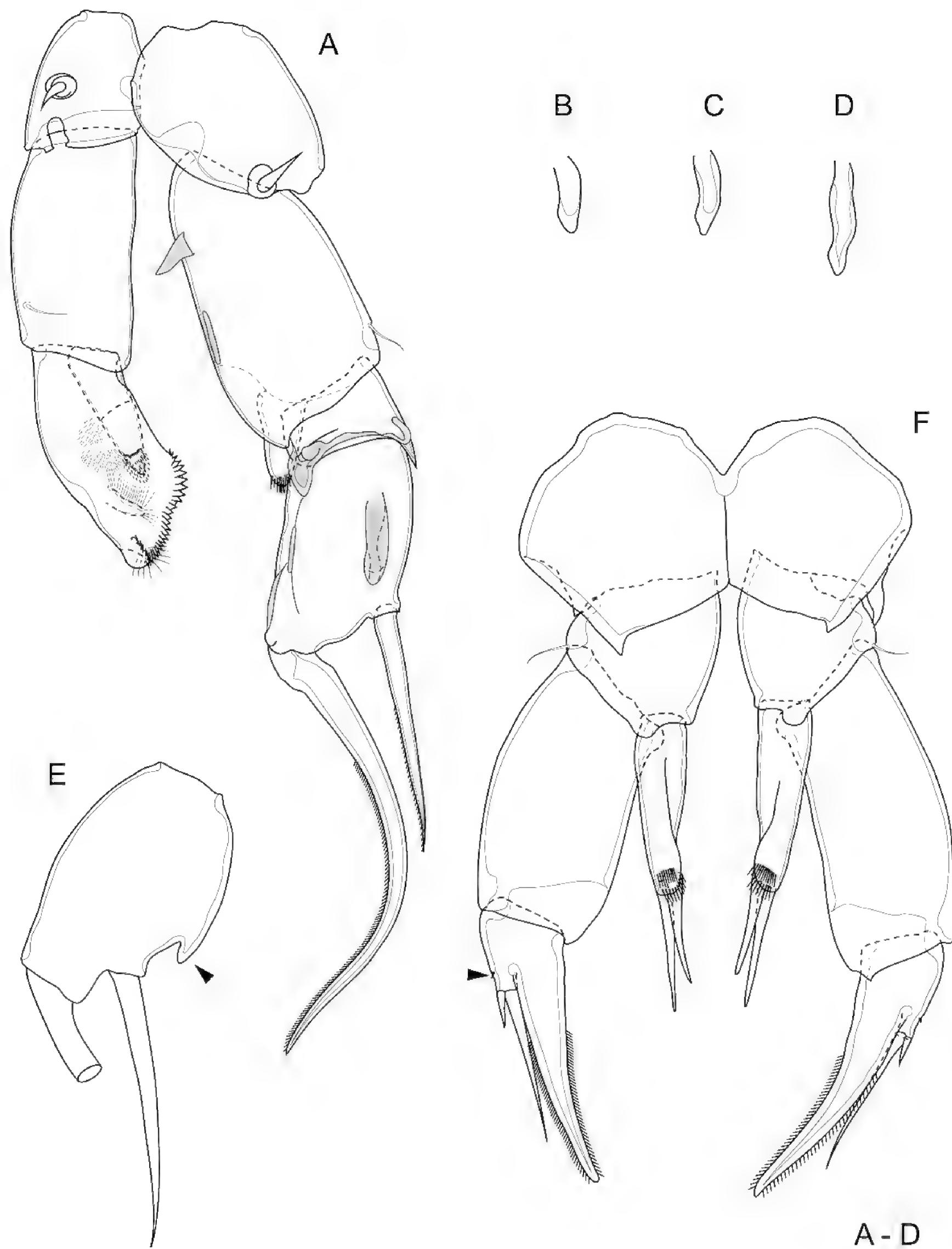


Figure 14. *Tropodiaptomus longiprocessus* sp. nov., male holotype. A. P5, dorsal view; B–D. Shape variation of hyaline lamella on exp-2 of right P5 in adult male, dorsal surface; E. Exp-2 of right P5 of the adult male specimen from the same population of holotype, lateral view (arrowhead indicates hyaline lamella); *Tropodiaptomus longiprocessus* sp. nov., female allotype: F. P5 (arrowhead indicates exp-3). Scale bar: 100 μ m.

Distribution and ecology. *Tropodiaptomus longiprocessus* sp. nov. was found only in its type locality. It is rare because it was found only in two of 471 samples collected from 206 freshwater habitats throughout Thailand between September 2017 and July 2019. Water temperature 26.6–28.0 °C, conductivity 623.3–

672.7 μ s cm⁻¹, salinity 0.3 ppt, total dissolved solids 385–424 mg L⁻¹, dissolved oxygen 2.5–6.0 mg L⁻¹, pH 7.2–7.3, and water depth 0.2–0.3 m, substrate with mud. However, in order to understand more in their habitat preference, the whole year samples are needed to be examined.

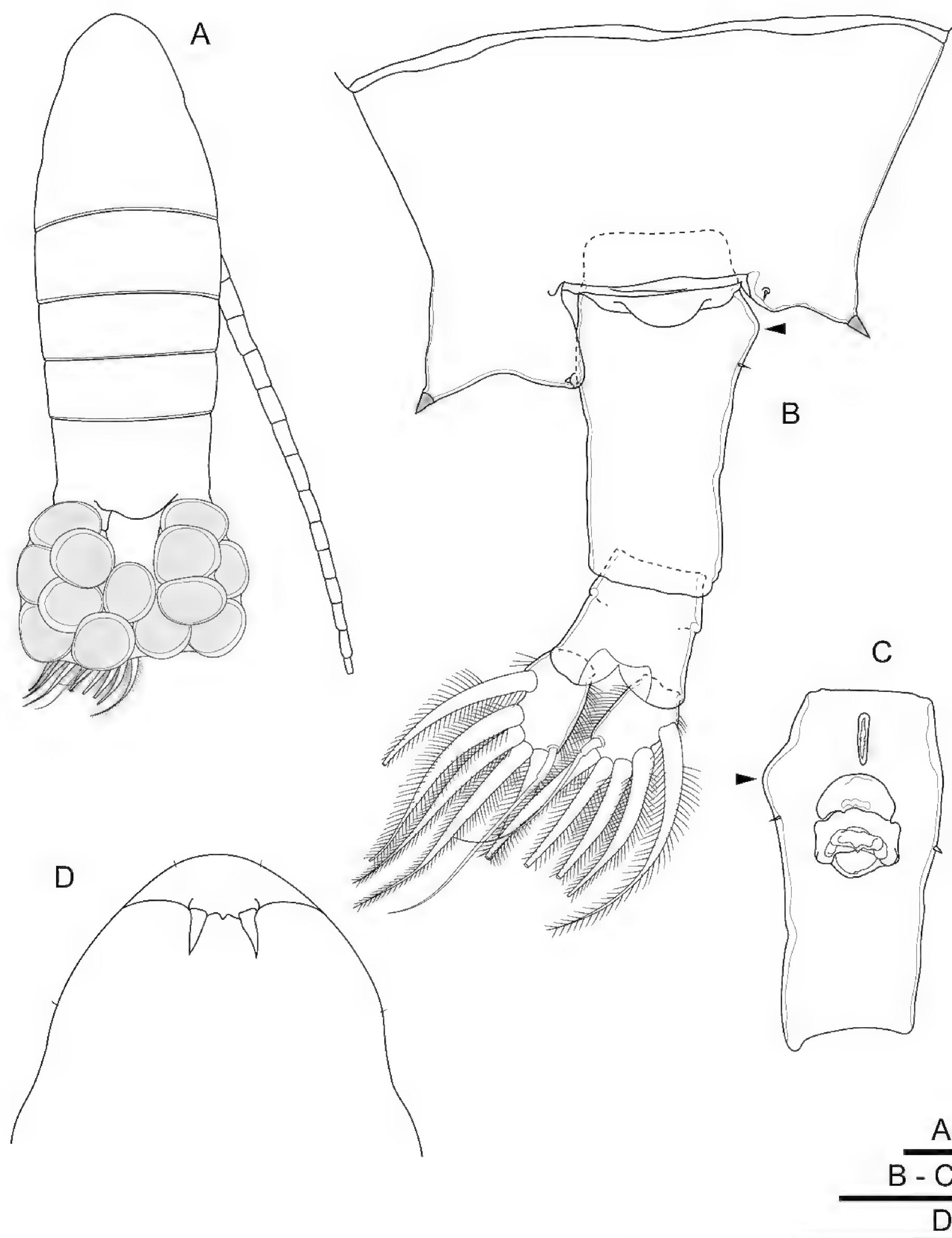


Figure 15. *Tropodiaptomus longiprocessus* sp. nov., female allotype. **A.** Habitus, dorsal view; **B.** Pediger 5 and urosome, dorsal view; **C.** Genital double-somite, ventral view; **D.** Rostrum. Scale bars: 100 µm.

Differential diagnosis. *Tropodiaptomus longiprocessus* sp. nov. differs from the congeneric species by the following respects: (i) antepenultimate segment of the male right antennule with straight spinous process reaching beyond the distal margin of next segment; (ii) inner margin of exopodal segment of the male left P5 with two lobes,

and with uniform serration; (iii) basis of the male right P5 with one triangular process and one longitudinal hyaline lamella; (iv) exp-1 of the male right P5 with triangular lobe on inner margin, distal outer corner produced into acute spinous process; and (v) exp-2 of the male right P5 rectangular, dorsal surface with two longitudinal hyaline

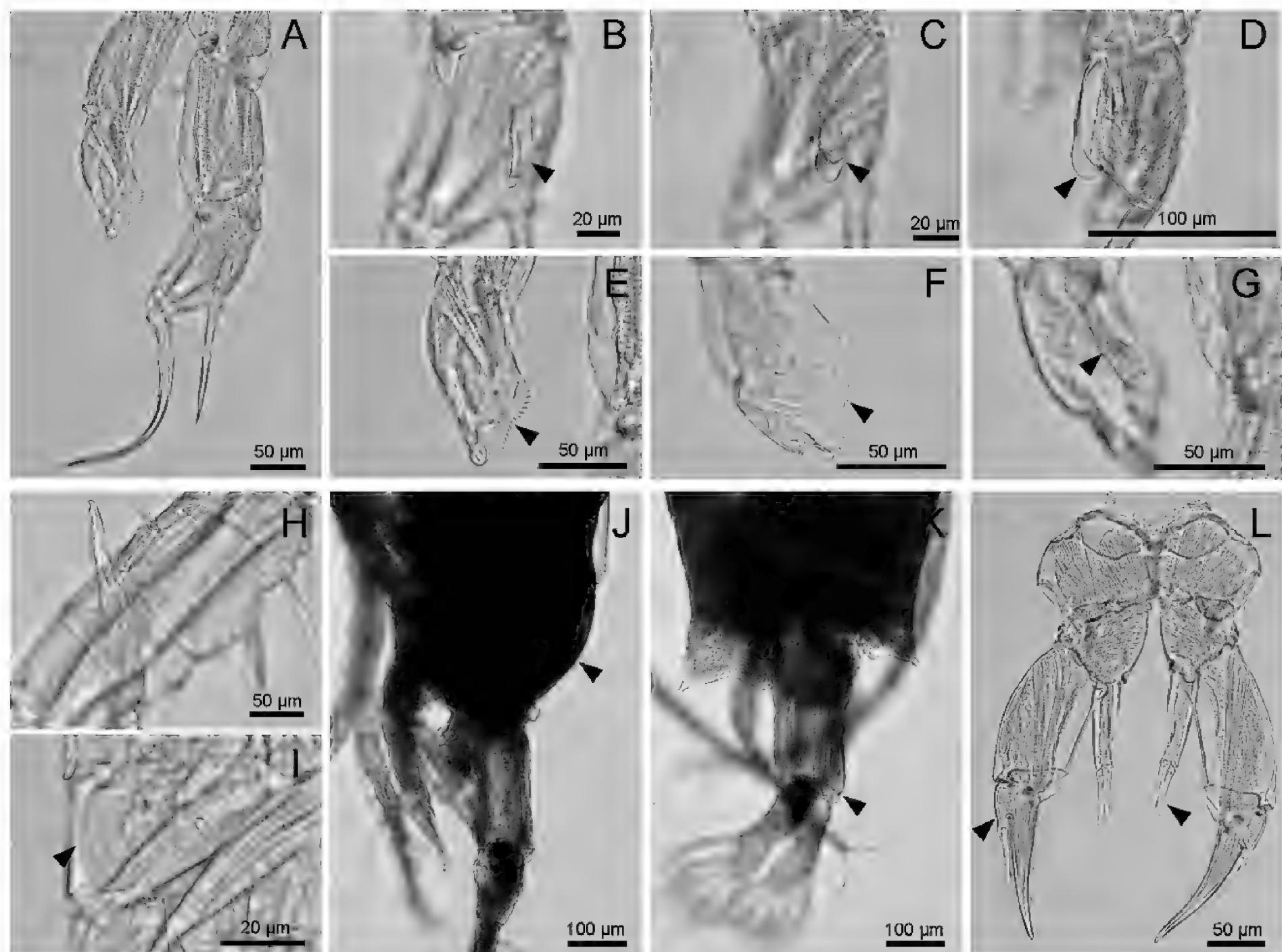


Figure 16. *Tropodiaptomus longiprocessus* sp. nov.; male. **A.** P5, dorsal view; **B, C.** Hyaline lamella on exp-2 of right P5, dorsal surface; **D.** Hyaline lamella on exp-2 of right P5, lateral surface; **E, F.** Exopodal segment of the male left antennule, dorsal surface; **G.** Exopodal segment of the male left antennule, ventral surface; **H.** Antepenultimate segment of right antennule; **I.** Lateral part of P1 basis; female; **J.** Pediger 5 and urosome, lateral view; **K.** Pediger 5 and urosome, dorsal view; **L.** P5. Arrowheads show the characters discussed in the description.

lamellae in middle and distal inner margin and one large and long process in middle in lateral third.

Tropodiaptomus longiprocessus sp. nov. is the most similar to *T. lanaonus*, but it differs distinctively in the following characters: (i) the dorsal surface of exp-2 of the adult male right P5 has two longitudinal hyaline lamellae and one large and long process; (ii) lateral wings of the fifth pediger of adult female are asymmetrical; (iii) anterior part of the genital double-somite of the adult female is dilated on the right side; (iv) right posterior corner of the genital double-somite of the adult female does not have a round lobe; and (v) distal end of the endopodal segment of the adult female P5 has smooth spiniform setae (Table 2).

The diversity of *Tropodiaptomus* in Thailand is presented as a pictorial key in Fig. 17 to facilitate easy and quick identification.

Tropodiaptomus species diversity and distribution in Thai water bodies

Tropodiaptomus species have been recorded from various ponds, lakes, streams, roadside canals and rice field

habitats in Thailand (Sanoamuang 2002; Saetang et al. 2020, 2022), with 12 species now recorded (Table 3) (Saetang et al. 2020, 2022; Sanoamuang and Dabseepai 2021). However, the status of some species remains unclear. Molecular data have confirmed that *T. cf. lanaonus* and *T. lanaonus* s. str. are different species (Saetang et al. 2022) but thorough morphological comparisons of numerous specimens are still needed to confirm species-level separation of *T. cf. lanaonus*. The validity of *T. doriai* is also in serious doubt, with no further reports on this species in Thailand since it was first reported by Daday in 1906 from a freshwater habitat close to Bangkok (Lai and Fernando 1981; Sanoamuang 2002).

Each species of *Tropodiaptomus* can be found in various types of freshwater habitats, while most occur in both temporary and permanent water bodies with a limited geographical distribution range (Fig. 18). Only *Tropodiaptomus vicinus* is widely distributed throughout Thailand, except in the north, while *T. oryzanus* is common but restricted to the northeastern region and *T. hebereri*, *T. megahyaline*, *Tropodiaptomus* sp. 1 and *Tropodiaptomus* sp. 2 have restricted distributions in one region in a few localities. Most species can be found

Tropodiaptomus

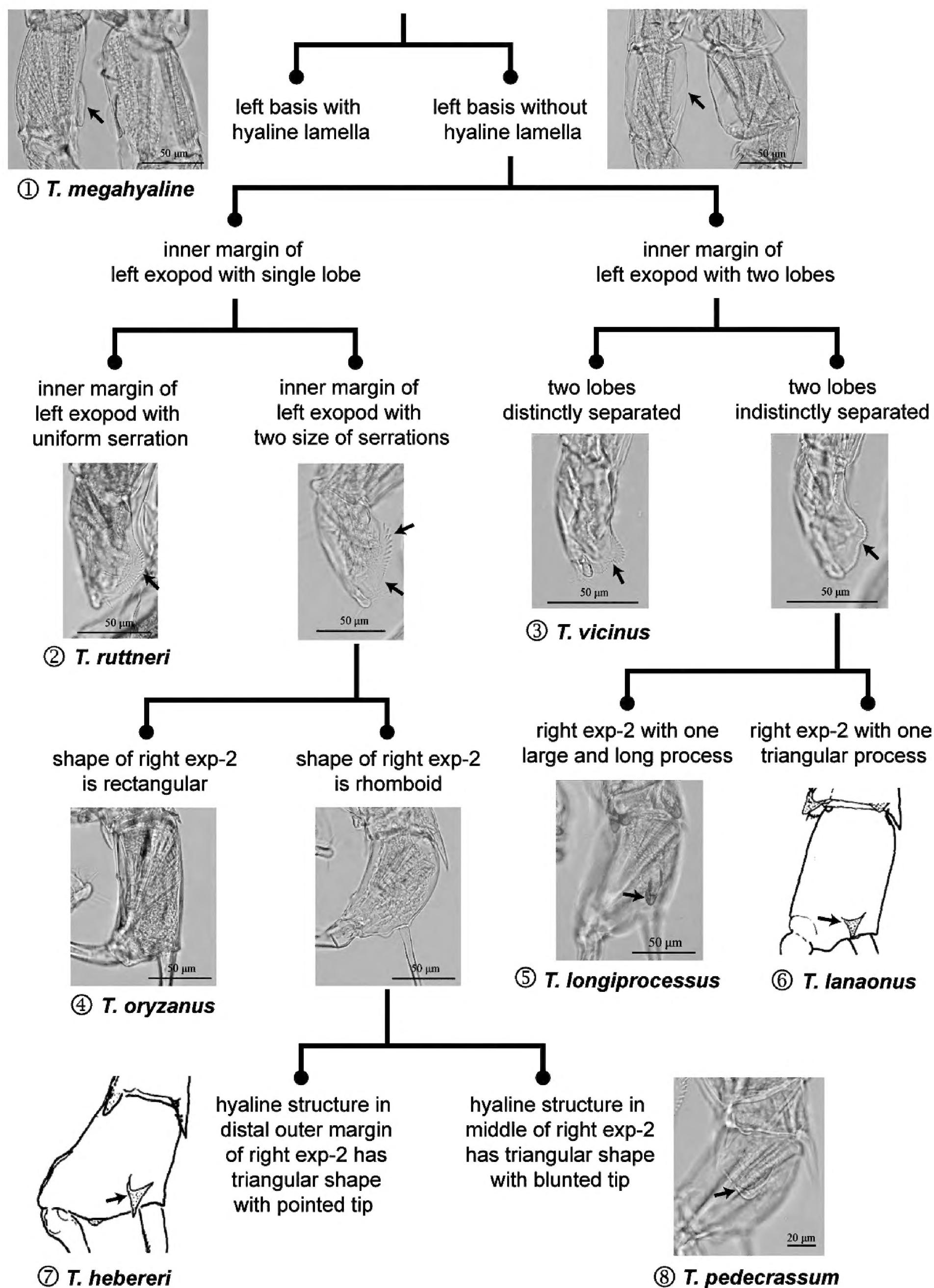


Figure 17. Pictorial key of the adult male P5 of *Tropodiaptomus* species found in Thailand. Right exp-2 of P5 of *Tropodiaptomus lanaonus* and *T. hebereri* were modified from Kiefer (1982).

during all seasons in Thailand. *T. oryzanus* and *T. vicinus* can be found all year round, in both rainy and dry seasons, including during low temperature periods in Northern Thailand. Some species are seasonal, with *T. pedecrassum* found only during low temperature periods in the north and *T. longiprocessus* and *Tropodiaptomus* sp.1 found only during the rainy season, with distribution restricted to temporary pools (Table 3).

Discussion

This study assessed the morphological characteristics and molecular data recorded by Saetang et al. (2020) and confirmed the species status of *Tropodiaptomus pedecrassum* sp. nov. and *Tropodiaptomus longiprocessus*

sp. nov. These two species clearly differ from their congeners: (i) the inner margin of the adult male left P5 had a single lobe and serration gradually decreased in size from the proximal to distal end in *Tropodiaptomus pedecrassum* sp. nov. with two lobes and uniform serration in *Tropodiaptomus longiprocessus* sp. nov. and (ii) exp-2 of the adult male right P5 was rhomboid-shaped, with one triangular-shaped process on the frontal surface in *Tropodiaptomus pedecrassum* sp. nov., and a rectangular shaped large and long process in the middle third lateral in *Tropodiaptomus longiprocessus* sp. nov.

The discovery of these two new species increases the number of *Tropodiaptomus* species recorded in Thailand from 10 to 12, thus accounting for 19% of species diversity worldwide. Based on the large number of samples taken throughout Thailand, most *Tropodiaptomus* species

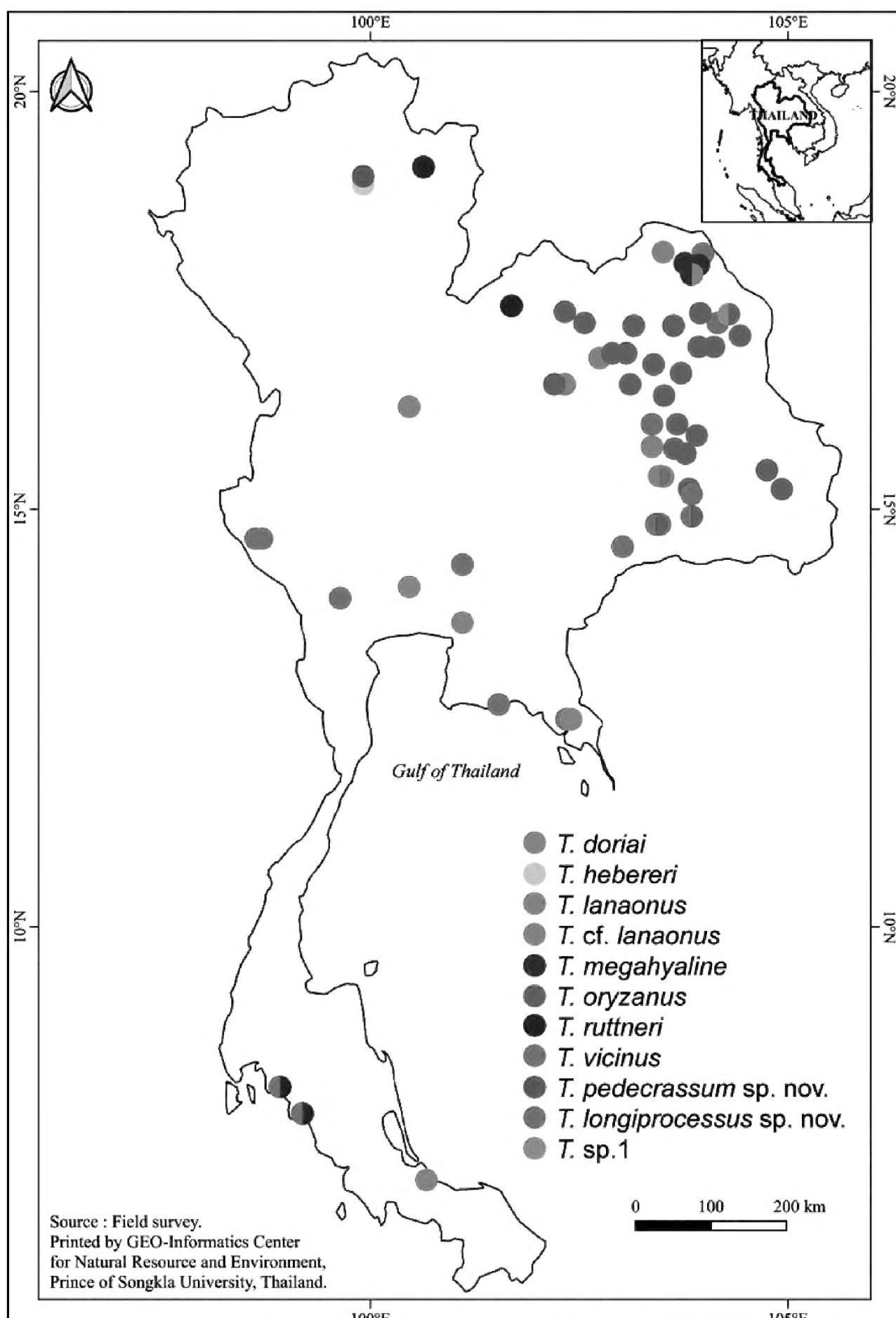


Figure 18. Distribution of *Tropodiaptomus* found in Thailand (*Tropodiaptomus* sp. 2 is not included in this figure because no distribution data are available).

Table 3. Geographic distribution of *Tropodiaptomus* species in Thai water bodies and remarks on their status.

Species	Distribution in Thai water bodies	Co-occurring species in Thai water bodies	Wider Distribution	Notes
1. <i>T. doriai</i>	Freshwater habitat close to Bangkok, central Thailand	No data	India, Indonesia and Sri Lanka (Kiefer 1982; Ambedkar 2012)	It was recorded only once in Thailand (Lai and Fernando 1981 referred to Daday 1906) and has never been found again. Therefore, its occurrence status is still doubtful.
2. <i>T. hebereri</i>	Cold season (January), in a roadside canal in northern Thailand	None	China, India, Indonesia and Malaysia (Kiefer 1930, 1982; Hsiao 1950; Ambedkar 2012)	
3. <i>T. lanaonus</i>	Floodplain, lake and pond in both dry (March) and rainy seasons (August) in central and northeastern Thailand	No data	Philippines (Kiefer 1982)	
4. <i>T. cf. lanaonus</i>	Rice field, river, roadside canal, swamp in both dry (May) and rainy seasons (June, September, October, November) in eastern, northeastern and southern Thailand	<i>Dentodiaptomus javanus</i> , <i>Eodiaptomus phuphanensis</i> , <i>Heliodiaptomus elegans</i> , <i>Mongolodiaptomus botulifer</i> , <i>M. dumonti</i> , <i>M. malaindosinensis</i> , <i>M. pectinidactylus</i> , <i>Phyllodiaptomus christineae</i> , <i>P. roietensis</i> , <i>P. surinensis</i> , <i>Phyllodiaptomus</i> sp., <i>Tropodiaptomus megahyaline</i> , <i>T. vicinus</i> , <i>Vietodiaptomus blachei</i>	–	This species does not agree with the original description of <i>T. lanaonus</i> in (i) the length of the spinous process in the antepenultimate segment of adult male right antennule is 1/2 to 3/4 of segment 21, and (ii) the ornamentation on the basis of adult male right P5 has one hyaline lamella and one apophysis or one hyaline lamella and no apophysis (Saetang et al. 2022)
5. <i>T. megahyaline</i>	Pond and rice field in both dry (May) and rainy season (June) in northeastern Thailand	<i>Heliodiaptomus elegans</i> , <i>Mongolodiaptomus malaindosinensis</i> , <i>M. pectinidactylus</i> , <i>Neodiaptomus songkramensis</i> , <i>Tropodiaptomus</i> cf. <i>lanaonus</i> , <i>Vietodiaptomus blachei</i>	Endemic in Thailand (Saetang et al. 2020)	
6. <i>T. oryzanus</i>	Several types of habitats including canal, floodplain, lake, man-made pond, pond, rice field and roadside canal in dry (April, May), rainy (June, August, October) and cold season (December) in northeastern Thailand	<i>Dentodiaptomus javanus</i> , <i>Neodiaptomus laii</i> , <i>Mongolodiaptomus botulifer</i> , <i>M. malaindosinensis</i> , <i>Tropodiaptomus vicinus</i>	Cambodia, China, Japan, Korea, Malaysia, Taiwan and Vietnam (Kiefer 1937, 1982)	
7. <i>T. ruttneri</i>	Peat swamp, swamp roadside canal and pond in both dry (March) and rainy seasons (August, November) in north, northeastern and southern Thailand	<i>Mongolodiaptomus botulifer</i>	China and Malaysia (Kiefer 1982)	
8. <i>T. vicinus</i>	Several types of habitats including canal, fish pond, floodplain, lake, man-made pond, marsh, peat swamp, pond, rice field, river, swamp and roadside canal in dry (April, May), rainy (June, August, October, November) and cold season (December) in the central, eastern, northeastern, southern and western Thailand	<i>Dentodiaptomus javanus</i> , <i>Eodiaptomus phuphanensis</i> , <i>Heliodiaptomus elegans</i> , <i>Mongolodiaptomus botulifer</i> , <i>M. malaindosinensis</i> , <i>Neodiaptomus laii</i> , <i>N.yangtsekiangensis</i> , <i>Phyllodiaptomus christineae</i> , <i>P. roietensis</i> , <i>P. surinensis</i> , <i>Tropodiaptomus oryzanus</i> , <i>T. cf. lanaonus</i>	Cambodia, India, Indonesia, Laos, Malaysia, Philippines and Vietnam (Lai et al. 1979; Kiefer 1982; Ambedkar 2012)	
9. <i>T. pedecrassum</i> sp. nov.	Swamp in cold season (January, February) in northern Thailand	<i>Mongolodiaptomus botulifer</i>		Recorded as <i>Tropodiaptomus</i> sp.1 in Saetang et al. (2022).
10. <i>T. longiprocessus</i> sp. nov.	Swamp in rainy season (June) in western Thailand	None		Recorded as <i>Tropodiaptomus</i> sp.2 in Saetang et al. (2022).
11. <i>Tropodiaptomus</i> sp. 1	Pond in rainy season (October) in northeastern Thailand	None		Recorded as <i>Tropodiaptomus</i> sp.3 in Saetang et al. (2022). Although the molecular data suggested that is a putative new species, more specimens are needed to prove its status.
12. <i>Tropodiaptomus</i> sp. 2	Pond in northern Thailand no information available on temporal distribution	No data		Recorded as <i>Tropodiaptomus</i> sp. in Sanoamuang and Dabseepai (2021).

have a limited distribution at one site or one region, with only *T. vicinus* having a wide distribution. Moreover, *Tropodiaptomus* is distributed in a specific habitat, temporary pools without vegetation and the occurrences of this genus are seasonal. This type of habitat is undersampled in SE Asia, however it is important for our understanding of the diversity of this genus. However, recent findings of new records in Southeast Asia (Defaye 2002; Ambedkar and Elia 2014; Saetang et al. 2020), including

the present discovery of two new species, indicate that this genus may have a wider geographical distribution than recorded previously (Lai and Fernando 1979b; Lai and Fernando 1981; Ambedkar and Elia 2014).

Saetang et al. (2022) considered that more undiscovered species exist in this genus in SE Asia. Further intensive study is required, in terms of intensive sampling year-round as well as the use of efficient tools such as molecular systematics together with morphological

examinations. This will clarify the taxonomic status of currently confusing data and more accurately estimate the species richness and ecological and biogeographical distribution of members of this genus in Thailand.

Acknowledgements

We would like to thank the editor and the reviewer for taking the time and effort to review the manuscript. We sincerely appreciate all the valuable comments and suggestions that helped us improve the quality of the manuscript. This research was approved by the Institutional Animal Care and Use Committee, Kasetsart University, Thailand (approval no. ACKU61-SCI-004). The authors would like to thank Kasetsart University for providing post-master research scholarship through the Biodiversity Center Kasetsart University. Dr. Rapeepan Jaturapruet is thankful for assistance in field sampling.

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